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Interseeding Native Warm-Season Grass Pastures

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I am submitting herewith a dissertation written by Jonathan D. Richwine entitled "Interseeding Native Warm-Season Grass Pastures." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Natural Resources.

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Interseeding Native Warm-Season Grass Pastures

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Jonathan Daniel Richwine
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DEDICATION

First and foremost, I dedicate this work to my Lord and Savior, Jesus Christ, for without Him none of this research could have taken place. “Then God said, ‘Let the earth bring forth grass, the herb that yields seed…on the earth’; and it was so.” Genesis 1:11. His love, mercy, and grace endure forever. “For God so loved the world that He gave His only begotten Son, that whoever believes in Him should not perish but have everlasting life.” John 3:16. If you are reading this and do not know Who I am talking about, HE knows you, loves you, and wants a relationship with you. “…for all have sinned and fall short of the glory of God, being justified freely by His grace through the redemption that is in Christ Jesus, whom God set forth as a propitiation by His blood, through faith, to demonstrate His righteousness, because in His forbearance God had passed over the sins that were previously committed, to demonstrate at the present time His righteousness, that He might be just and the justifier of the one who has faith in Jesus.” Romans 3:23-26. “...God our Savior, who desires all men to be saved and to come to the knowledge of the truth. For there is one God and one Mediator between God and men, the Man Christ Jesus, who gave Himself a ransom for all...” 1 Timothy 2:3-6.

Next, I dedicate my dissertation to my loving and supportive family: Aundrea, Ian, Jim, Frances, Kadie, Jeremy, Maddie Claire, Maverick, Lillie Kate, Cile, Ronald, Ronnie, Hannah, Dustin, and Lynley, as well as close friends. Y’all have encouraged and influenced me in more ways than you realize and will ever know. Lastly, this is dedicated to those students and researchers who will come after me. Don’t let your work become your idol. Just breathe and Godspeed! Proverbs 16:9.

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ABSTRACT

Cool-season annual (CSA) grass and legume species, as well as warm-season forbs, can enhance established native warm-season grass (NWSG) pastures by extending the grazing season, reducing supplemental feed costs, suppressing weeds, increasing herbage production and overall forage quality, and increasing food and cover resources for pollinators and wildlife. Therefore, two NWSG experiments were conducted near Spring Hill, TN, 2018-2020, to assess three CSA seeding options (cereal rye monoculture, a cereal rye, 'Purple Top' turnip, 'Trophy' rape, 'Frosty' berseem clover, and 'Dixie' crimson clover polyculture, or non-planted control) and two warm-season N rates (0 or 67 kg N ha^{-1}) on established switchgrass (SG) and big bluestem/indiangrass (BBIG) pastures. Switchgrass and BBIG plant density were not influenced by CSA seeding but varied by Year ($P < 0.001$). Plant density for BBIG was lower in 2020 than 2018 (23.6 vs. 29.1 plants m^{-2}, respectively), whereas SG plant density was similar for 2018 and 2020 (15.0 and 14.0 plants m^{-2}, respectively). Hay yield for both SG and BBIG varied among harvests (July 2019, July 2020, and September 2020; $P < 0.001$) but not by CSA seeding. Additionally, two other NWSG experiments were conducted near Greeneville, TN, 2017-2020, to assess the effect of within-season rest on the persistence of 11 native forbs when interseeded into established SG and BBIG pastures. Within-season rest treatment was not influential for total forb plant density or NWSG tiller density thus indicating persistence of forbs may not require within-season rest. Purple prairie clover never established while Illinois bundleflower was only observed flowering once despite having the greatest seeding rate among the forbs. Of the 11-species in the current

mixture, interseeding a 6-species polyculture of black-eyed susan, Dixie ticktrefoil, lanceleaf coreopsis, Maximilian sunflower, oxeye sunflower, and purple coneflower could allow for plant biodiversity while offering floral resources for pollinators during the NWSG grazing season. Because CSA and native forbs did not affect plant density over the course of these experiments, incorporating CSA or native forbs may be a viable option for increasing grazing opportunities and forage production in NWSG pastures.

TABLE OF CONTENTS

INTRODUCTION 1
Overview of Warm-Season Grasses 2
Overview of Native Warm-Season Grasses 3
Companion Crops 7
REFERENCES 11
CHAPTER I - INTERSEEDING WINTER ANNUALS INTO NATIVE WARM-SEASON GRASS PASTURES 16
ABSTRACT 17
INTRODUCTION 18
MATERIALS AND METHODS 21
Site Description 21
Experimental Design 22
Animal Management and Measurements 23
Pasture Management and Measurements 24
Nutritive Value Analysis 25
Statistical Analysis 26
RESULTS AND DISCUSSION 27
Environmental Conditions 27
Density 28
Hay Yield, Nutritive Value, and Grazing Days 31
CONCLUSIONS 33
REFERENCES 34
APPENDIX I 38
CHAPTER II - NATIVE FORBS INTERSEEDED INTO NATIVE GRASS PASTURES PERSIST UNDER GRAZING 44
ABSTRACT 45
INTRODUCTION 46
MATERIALS AND METHODS 48
Site Description 48
Experimental Design 49
Animal Management and Measurements 50
Pasture Management and Measurements 51
Nutritive Value Analysis 52
Statistical Analysis 53
RESULTS 54
Environmental Conditions 54
Tiller and Plant Density 54
Switchgrass 54
Big Bluestem/Indiangrass 56
Forage Mass and Nutritive Value 58
Switchgrass 58
Big Bluestem/Indiangrass 58
Native Forb Flowering 59

Switchgrass 59
Big Bluestem/Indiangrass 60
Steer Performance and Pasture Productivity 60
Switchgrass 60
Big Bluestem/Indiangrass 60
DISCUSSION 61
Tiller and Plant Density 61
Forage Mass and Nutritive Value 64
Native Forb Flowering 65
Steer performance 66
CONCLUSIONS 67
REFERENCES 69
APPENDIX II 73
CONCLUSIONS 99
APPENDIX III - USING A BROWNTOP MILLET COMPANION CROP TO AID NATIVE GRASS ESTABLISHMENT 100
ABSTRACT 101
INTRODUCTION 102
MATERIALS AND METHODS 104
Site Description 104
Experimental Design 105
Data Collection 106
Statistical Analysis 107
RESULTS 108
Environmental Conditions 108
Big Bluestem 109
Establishment-year Plant Density 109
Biomass Dry Matter Yield 110
Switchgrass 110
Establishment-year Plant Density 110
Biomass Dry Matter Yield 111
Browntop Millet 111
Discussion 112
Establishment-year Plant Density 112
Biomass Dry Matter Yield 115
CONCLUSIONS 117
ACKNOWLEDGEMENTS 118
REFERENCES 119
APPENDIX IIIA 123
VITA 132

LIST OF TABLES

Table 1.1. Grazing days ha^{-1} for cow-calf pairs grazing interseeded cereal rye monoculture (RYE) and cereal rye, crimson clover, berseem clover, turnip, and rape polyculture (Poly) within switchgrass and big bluestem/indiangrass pastures, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN. 38

Table 1.2. Plant cover (% m^{-2}) of cool-season annual treatments of interseeded cereal rye and polyculture (cereal rye, turnip, rape, berseem clover, and crimson clover) within switchgrass and big bluestem/indiangrass pastures prior to grazing, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN. 38

Table 1.3. Cool-season annual plant density (plants m^{-2}) of interseeded cereal rye and polyculture (cereal rye, turnip, rape, berseem clover, and crimson clover) within switchgrass and big bluestem/indiangrass pastures prior to grazing, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN. 39

Table 1.4. Mean crude protein (CP), acid detergent fiber (ADF), amylase neutral detergent fiber (aNDF), and *in vitro* true dry matter digestibility 48 hours (IVTDMD48H) of interseeded cereal rye (RYE) and polyculture (cereal rye, turnip, rape, berseem clover, and crimson clover) within switchgrass and big bluestem/indiangrass pastures prior to grazing, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN. 39

Table 1.5. Mixed-effects ANOVA model for switchgrass and big bluestem/indiangrass plant density (plants m^{-2}) and switchgrass tillers ($plant^{-1}$) in native pasture grazing experiments, 2018-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN. 40

Table 1.6. Mixed-effects ANOVA model for hay yield (Mg ha^{-1}) by cool-season annual (CSA) pasture for switchgrass and big bluestem/indiangrass pastures in native pasture grazing experiment, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN. 40

Table 1.7. Mixed-effects ANOVA model for nutritive value parameters for hay samples by cool-season annual (CSA) pasture for switchgrass and big bluestem/indiangrass pastures in native pasture grazing experiment, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN. 41

Table 2.1. Annual soil tests (0-15 cm; Mehlich 1) for switchgrass and big bluestem/indiangrass pastures planted with an 11-species forb blend at the

University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN, in 2018-2020. 73

Table 2.2. Native warm-season forbs species planted in a mixture for native grass pasture grazing experiments, June 2017, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 73

Table 2.3. Grazing treatment dates within switchgrass and big bluestem/indiangrass pastures for native grass pasture grazing experiments, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 74

Table 2.4. Sampling period dates for grazing treatments within switchgrass and big bluestem/indiangrass pastures for native grass pasture grazing experiments planted with an 11-species forb blend, 2019-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 74

Table 2.5. Mixed-effects ANOVA model for switchgrass tiller density and plant density of 11 native forb species within native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 75

Table 2.6. Black-eyed susan mean plant density (plants m^{-2}) in a switchgrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 76

Table 2.7. Mean plant density (plants m^{-2}) for DITI, LANC, and PPEA for grazing treatment by year in a switchgrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 77

Table 2.8. Mean plant density (plants m^{-2}) for DITI, LANC, OXEY, PLAC, and PPEA for period by year in a switchgrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 78

Table 2.9. Forb species overall rank for abundance, persistence, and flowering for 11 native forb species planted within switchgrass and big bluestem/indiangrass pastures for native grass pasture grazing experiments, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 79

Table 2.10. Mixed-effects ANOVA model for big bluestem/indiangrass tiller density and plant density of 11 native forb species for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 80

Table 2.11. Mean plant density (plants m^{-2}) for PCON for grazing treatment by year in a big bluestem/indiangrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 81

Table 2.12. Mean plant density (plants m^{-2}) for BESU and MAXI for grazing treatment by period in a big bluestem/indiangrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 81

Table 2.13. Mean plant density (plants m^{-2}) for BESU, LANC, OXEY, PCON, and PLAC for period by year in a big bluestem/indiangrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 82

Table 2.14. Mixed-effects ANOVA model for forb, switchgrass, and total forage mass across within-season rest treatments sampled within sampling date in a switchgrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 83

Table 2.15. Mixed-effects ANOVA model for forb, big bluestem/indiangrass, and total forage mass across within-season rest treatments sampled within sampling date in a big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 84

Table 2.16. Mixed-effects ANOVA model for forage nutritive value parameters across within-rest treatments sampled within sampling date in a big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 85

Table 2.17. Chi-square test for native forb flowering of an 11-species forb blend planted within switchgrass and big bluestem/indiangrass pastures for native grass pasture grazing experiments, 2019-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. 86

Table 3A.1. Harvest dates for browntop millet defoliation strategies for big bluestem and switchgrass at each site at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN, during establishment experiments in 2016 and 2017. 123

Table 3A.2. Mixed-effects ANOVA model results for establishment-year plant density of big bluestem seedlings, weeds, and browntop millet (BTM) + weeds at each site at

East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN, 2016-2017, during a big bluestem establishment experiment. 124

Table 3A.3. Mixed-effects ANOVA model results for big bluestem and switchgrass second-year biomass dry matter yield for each site at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN, during establishment experiments. Harvests were conducted in 2017 and 2018 for Site 1 and 2, respectively. 125

Table 3A.4. Mixed-effects ANOVA model results for establishment-year plant density of switchgrass seedlings, weeds, and browntop millet (BTM) + weeds at each site at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN, 2016-2017 during a switchgrass establishment experiment. 126

LIST OF FIGURES

Figure. 1.1. Field layout of three cool-season annual (CSA) treatments (non-planted control, cereal rye monoculture, and CSA polyculture of cereal rye, ‘Purple Top’ turnip, ‘Trophy’ rape, ‘Frosty’ berseem clover, and ‘Dixie’ crimson clover) coupled with two warm-season N fertilization rates (0 and 67 kg N ha^{-1}) within 14, 1.2-ha pastures (eight switchgrass and six big bluestem/indiangrass mixture) at the University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN, 2018-2020. 42

Figure. 1.2. (a) Mean monthly air temperature (°C) and 30-year mean and (b) total monthly precipitation (mm) and 30-year mean for the University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN, 2018-2020. †Some months’ data are missing in overall 30-year mean from 1991-2020. 43

Figure 2.1 Field layout of five grazing treatments based on the timing of within-season rest (no rest, early rest, middle rest, late rest, and no grazing control) arranged in a completely randomized design with four replicates (n = 20 plots) within each of the two, 1.2-ha pastures (switchgrass and big bluestem/indiangrass mixture) interseeded with an 11-species biodiversity mixture of native forbs at the University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN, 2017-2020. 87

Figure. 2.2. (a) Mean monthly air temperature (°C) and 30-year mean and (b) total monthly precipitation (mm) and 30-year mean for University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN, 2017-2020. †Some months’ data are missing in overall 30-year mean from 1991-2020. . 88

Figure 2.3. Mean tiller density (tillers m^{-2}) for switchgrass by period within year (2018-2020) at the University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. *Different letters indicate significant difference at $\alpha = 0.05$ for Year x Period interaction (Fisher’s least significant difference). 89

Figure 2.4. Mean tiller density (tillers m^{-2}) for big bluestem/indiangrass by period within year (2018-2020) at the University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. *Different letters indicate significant difference at $\alpha = 0.05$ for Year x Period interaction (Fisher’s least significant difference). 90

Figure 2.5. Mean big bluetem/indiangrass dry matter yield (Mg ha^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, $\alpha = 0.05$ 91

Figure 2.6. Mean total forage dry matter yield (Mg ha^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, $\alpha = 0.05$ 92

Figure 2.7. Mean crude protein (g kg^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, $\alpha = 0.05$ 93

Figure 2.8. Mean amylase neutral detergent fiber (g kg^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, $\alpha = 0.05$ 94

Figure 2.9. Mean acid detergent fiber (g kg^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, $\alpha = 0.05$ 95

Figure 2.10. Mean *in vitro* true dry matter digestibility 48 hours (g kg^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, $\alpha = 0.05$ 96

Figure 2.11. Flowering percentage of 10 native warm-season forb species pooled across years (2019-2020) at the same sampling periods in a switchgrass pasture for native grass pasture grazing experiment at the University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. †Flowering percentage compared across grazing treatments within sampling periods. All sampling periods compared no graze and no rest to the respective sampled grazing treatment. May = early rest, middle rest, and late rest; June = early rest; July = middle rest; July/Aug = late rest; Aug/Sept = early rest, middle rest, and late rest.. 97

Figure 2.12. Flowering percentage of 10 native warm-season forb species pooled across years (2019-2020) at the same sampling periods in a big bluestem/indiangrass

pasture for native grass pasture grazing experiment at the University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. †Flowering percentage compared across grazing treatments within sampling periods. All sampling periods compared no graze and no rest to the respective sampled grazing treatment. May = early rest, middle rest, and late rest; June = early rest; July = middle rest; July/Aug = late rest; Aug/Sept = early rest, middle rest, and late rest. 98

Figure 3A.1. (a) Mean monthly air temperature (°C) and 30-year mean and (b) total monthly precipitation (mm) and 30-year mean for East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN, 2016-2018. †Some months' data are missing in overall 30-year mean from 1988-2018. ‡No data were reported in 2016.. 127

Figure 3A.2. Establishment-year plant density (seedlings m^{-2}) for big bluestem (BB), browntop millet (BTM), and weeds by BTM seeding rate (kg PLS ha^{-1}) at 30 and 60 days after planting (DAP) BB for Site 1 (top) and Site 2 (bottom) at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN. †Number of BB seedlings per BTM seeding rate at 30 and 60 DAP. ‡Different lowercase letters indicate significant differences among weed + BTM seedling totals by BTM seeding rates at 30 and 60 DAP within site. §Different UPPERCASE letters indicate significant differences for BB seedlings by BTM seeding rate at 30 and 60 DAP within site.. 128

Figure 3A.3. Establishment-year plant density (seedlings m^{-2}) at dormancy for big bluestem (BB) and switchgrass (SG) by browntop millet (BTM) seeding rate (kg PLS ha^{-1}) compared to using imazapic (Plateau) for Site 1 (2016) and Site 2 (2017) at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN. Plant density for imazapic treatment (BB only) are horizontal lines; not compared statistically to other BB treatments. †Different UPPERCASE letters indicate significant differences for BB plant density by BTM seeding rate within site. ‡Different lowercase letters indicate significant differences for SG plant density by BTM seeding rate within site. 129

Figure 3A.4. Biomass dry matter (DM) yield (Mg DM ha^{-1}) for (a) big bluestem and (b) switchgrass by browntop millet (BTM) seeding rate (kg PLS ha^{-1}) following the second year of each study at Site 1 (2017) and Site 2 (2018) at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN. †Different UPPERCASE letters indicate significant differences among Site 1 (2017) DM biomass yield among BTM seeding rate per species. ‡Different lowercase letters indicate significant differences among Site 2 (2018) DM biomass yield among BTM seeding rate per species.. 130

Figure 3A.5. Establishment-year plant density (seedlings m^{-2}) for switchgrass (SG), browntop millet (BTM), and weeds by BTM seeding rate (kg PLS ha^{-1}) at 30 and 60 days after planting (DAP) SG for Site 1 (top) and Site 2 (bottom) at East Tennessee

AgResearch and Education Center-Plant Science Unit, Knoxville, TN. †Number of SG seedlings per BTM seeding rate at 30 and 60 DAP. ‡Different lowercase letters indicate significant differences among weed + BTM seedling totals by BTM seeding rate at 30 and 60 DAP within site. §Different UPPERCASE letters indicate significant differences for SG seedlings by BTM seeding rate at 30 and 60 DAP within site. 131

INTRODUCTION

OVERVIEW OF WARM-SEASON GRASSES

Warm-season (C_4) grasses (WSG) are from the Poaceae family that conduct photosynthesis via C_4 photosynthetic pathway. Most WSG are located between 30°N and 30°S latitude (Moser et al., 2004), where the most favorable temperatures for WSG growth occurs. However, many WSG species are adapted to areas with a temperate climate (Waramit, Moore, & Heggenstaller, 2011), such as the dominant species of the tall grass prairie of North America (Weaver, 1954). The optimum temperature for growth of C_4 grasses is from 30 to 35°C (Long, 1999). In Tennessee, WSG grow predominantly during late spring, summer, and early fall (Ball et al., 2015).

Warm-season grasses have multiple characteristics that make them more advantageous to grow during summer months rather than cool-season (C_3) grasses (CSG). Warm-season grasses can produce the same amount of dry matter (DM) as that of CSG while utilizing one-third to one-half less water (Moser et al., 2004). Although C_4 grasses have a greater water-use efficiency (WUE) than C_3 grasses, not all WSG are drought tolerant (Moser et al., 2004). Also, WSG are able to use nitrogen (N) more efficiently than C_3 grasses thus reducing the cost and amount of N inputs needed in a forage production system. However, N fertilization is still required to optimize forage yield (Brejda, 2000).

Forage production in the Mid-South focuses on introduced, exotic grass species. Bermudagrass [*Cynodon dactylon* (L.) Pers.] and annual species supply the majority of warm-season forage in the Mid-South (Burns et al., 1984) while tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] is the main CSG used in Tennessee (Bates,

1997) and in the Mid-South (Steen et al., 1979; Pendulum et al., 1980). During the warmer months of the year in Tennessee, CSG become semi-dormant. This period is referred to as the “summer slump”. Some producers stockpile CSG by not grazing or reducing stocking rates during spring in these pastures to extend cool-season grazing further into the summer. However, this management approach allows the grass to mature therefore reducing its nutritive value (Moore et al., 2004). By relying on both WSG and CSG, a greater overall forage nutritive value can be achieved (Keyser, Bates, Waller, Harper, & Holcomb. 2011b). Also, having grass species from both photosynthetic pathways can aid in extending the number of grazing days, thus lowering the amount and number of days of supplemental feeding (Hoveland et al., 1977). By incorporating WSG into rotational stocking management with CSG, CSG are allowed time to recover following grazing and can be rested when growth conditions are unfavorable (Moore et al., 2004).

OVERVIEW OF NATIVE WARM-SEASON GRASSES

Native warm-season grasses (NWSG) are species that grow during the warmer months and inhabited North America prior to European settlement. The perennial, deep-rooted, bunchgrass species were dominant in the tallgrass prairie of the United States. However, multiple species, such as those used for forage production, ranged farther east than the tallgrass prairie to encompass the Mid-South and Southeastern United States. Native WSG species are known for their adaptability to environmental conditions associated with the Mid-South. These conditions include (but are not limited to) insect, disease, heat, and drought tolerance (Keyser, Bates, Waller, Harper, & Holcomb. 2011a).

Native WSG are utilized for forage and biomass production, wildlife habitat restoration, riparian buffers, roadside revegetation efforts, and controlling soil erosion. Some NWSG that have received attention as forage crops include big bluestem [*Andropogon gerardii* Vitman], eastern gamagrass [*Tripsacum dactyloides* (L.) L.], indiangrass [*Sorghastrum nutans* (L.) Nash], little bluestem [*Schizachyrium scoparium* (Michx.) Nash], and switchgrass [*Panicum virgatum* L.].

Native WSG require proper nutrient management for optimum growth. The most limiting nutrient in NWSG production is N (Vogel et al., 2002). Nitrogen is needed to produce large amounts of high-quality switchgrass (Smith, 1979; Hall et al., 1982). Even though C_4 grasses have a higher N use efficiency than C_3 grasses, WSG normally utilize less than 60% of N from applied fertilizers (Sinclair, 2006). When fertilizing switchgrass, applying a minimum of 50 kg N ha^{-1} should occur during the first year of production (year 2, the year following establishment). Following the first year of production, application of 67-100 kg N ha^{-1} annually has been recommended (Wolf & Fiske, 1995; McLaughlin & Walsh, 1998; Mooney et al., 2009). Vogel et al. (2002) found that 120 kg N ha^{-1} was the optimum N fertilization rate when harvesting switchgrass twice for forage in Iowa and Nebraska. Muir et al. (2001) reported that switchgrass reached maximum biomass production or began to plateau when fertilized with 168 kg N ha^{-1} in Texas.

However, even with marginal inputs or when grown on poor sites, these grasses can be productive. Berg (1995) reported that unfertilized swards of a NWSG mixture consisting of blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths], indiangrass, little bluestem, sand bluestem [*Andropogon hallii* Hack], sideoats grama

[*Bouteloua curtipendula* (Michx.) Torr.], and switchgrass grown in northwestern Oklahoma yielded an average of 1.2 Mg ha^{-1} yr^{-1} of herbage over three years. He also noted that NWSG yield increased by 15 kg with each kg of N applied over the three-year study. Gillen and Berg (1998) reported 60 and 63% increase in forage mass in June and August, respectively, when an ungrazed NWSG mixture consisting of blue grama, sideoats grama, indiangrass, little bluestem, sand bluestem, and switchgrass was fertilized with 35 kg N ha^{-1} yr^{-1} as compared to the unfertilized control. Jung et al. (1985) conducted a grazing experiment in Pennsylvania with switchgrass and big bluestem cultivars from 1975-1977. Native WSG were planted in 1973 into a prepared seedbed, grazed three times a year for three years (1975-1977), and no N fertilizer was added to the plots until 1977. This study shows how these grasses can be managed with low fertilizer inputs. Currently, 67 kg N ha^{-1} is the recommended rate for SG forage production (Keyser et al., 2011b; Popp et al., 2018).

Big bluestem and indiangrass are commonly planted in polycultures with one another (Keyser, Harper, Bates, Waller, & Holcomb, 2011c). Early summer biomass production of big bluestem and indiangrass normally has greater nutritive value than switchgrass (Gillen et al., 1998; Mitchell et al., 2001; McIntosh et al., 2015). Hall et al. (1982) found that big bluestem had greater season-long DM yield (6.77 Mg ha^{-1}) than switchgrass and indiangrass (6.33 and 5.73 Mg ha^{-1}, respectively) when harvested four times and averaged across four replications and three N rates in Iowa; switchgrass yield exceeded that of indiangrass. Griffin et al. (1980) studied the quality of big bluestem, switchgrass, and tall fescue. They determined that WSG dry matter intake and dry matter digestibility are equal to or better than that of tall fescue when tall fescue was harvested in summer or fall.

Multiple researchers (Burns & Fisher, 2013; Keyser et al., 2016; Backus et al., 2017) have conducted grazing studies on NWSG to evaluate their ability to produce weight gain in cattle (*Bos taurus*). Cattle grazing big bluestem and indiangrass mixed stands can attain 1.2-2.0 times greater season long average daily gains (ADG) than those grazing other NWSG (Keyser et al., 2011a). Krueger and Curtis (1979) found ADG of 1.08, 0.93, 0.88, and 0.70 kg day^{-1} when yearling steers were grazing indiangrass, switchgrass, sideoats grama, and big bluestem, respectively, in a study conducted in South Dakota with total beef weight gains of 119, 146, 112, and 138 kg ha^{-1}, respectively. Burns and Fisher (2013) reported that steers grazing three NWSG (big bluestem, switchgrass, and eastern gamagrass) in North Carolina had greater ADG (1.08, 0.91, and 0.87 kg day^{-1}, respectively) than a bermudagrass-tall fescue system (0.73 kg day^{-1}). They also reported a total beef yield of 839, 752, and 732 kg ha^{-1} for animals grazing switchgrass, eastern gamagrass, and big bluestem, respectively. Working in Iowa, Moore et al. (2004) found across five grazing seasons that big bluestem generally had greater digestibility than switchgrass whereas switchgrass pastures generally had greater available forage and crude protein (CP). Moore et al. (2004) also reported that beef cattle grazing big bluestem following smooth bromegrass in Iowa in 1997 had greater live weight gains than those animals grazing CSG during the same time of year.

COMPANION CROPS

Companion crops, crops planted with or within a species to increase or hasten returns of an area of land, can be advantageous in a forage production system. Cool-season annual grasses and legumes have been reported to increase the number of grazing days per season (Hoveland et al., 1977) and lower production costs by reducing the need for supplemental feedstuffs when incorporated into warm-season pastures. Adding legumes to a grazing system can have positive effects from both environmental and ecological perspectives. Legumes enhance the soil by contributing carbon from green manure and organic N (Ashworth et al., 2015). Legumes have Rhizobium bacteria that inhabit nodules on the roots and fix atmospheric N into a usable form for plants (Graham, 2005; Peoples et al., 2009), which in turn can reduce N inputs (Ashworth et al., 2015). Legumes also reduce the contamination of groundwater by soil nitrates (NO_3) and aid in decreasing weed encroachment by increasing ground cover (Ashworth et al., 2015). These companion crops compete for the same nutrients, sunlight, and water as the weeds thereby decreasing the resources used by invasive species (Ashworth et al., 2015).

Cool-season legumes planted into WSG pastures can extend the grazing season (Springer, 1997), by increasing CP, forage intake, and overall animal performance (Blaser et al., 1976; Vallis, 1976; Van Soest, 1976; Marten, 1985), while also increasing forage production. George et al. (1995) concluded that incorporating cool-season legumes into switchgrass pasture could be beneficial to livestock producers for such reasons. Bow et al. (2008) reported that switchgrass-arrowleaf clover and switchgrass-common vetch [*Vicia sativa* L.] mixed plots grown without compost in central Texas produced an average of 60% greater forage than switchgrass monocultures in the second year of the study.

Incorporating warm-season legumes into a mixture with WSG can be advantageous as well. Posler et al. (1993), working in Kansas, evaluated 18 binary legume-grass mixtures and reported that all of them except for a leadplant [*Amorpha canescens* Pursh]-switchgrass mixture had greater forage yield and CP than monocultures of indiangrass, sideoats grama, and switchgrass. Dovel et al. (1990) researching legume-grass mixtures in eastern Texas noted that a binary mixture of Illinois bundleflower [*Desmanthus illinoensis* (Michx.) MacMill. ex B.L. Rob. & Fernald] and kleingrass [*Panicum coloratum* L.] produced more forage than a monoculture of kleingrass.

Species compatibility should be considered when intercropping multiple plants. Both non-leguminous and leguminous forb species can inhibit adjacent plant growth. Wagner (2020) reported that black-eyed susan [*Rudbeckia hirta* L.], an annual/biennial forb species, dominated an 18-species mixture the first two years after planting. However, by the third year, Wagner (2020) reported a dominance shift to perennial species (e.g., grey-headed coneflower [*Ratibida pinnata* (Vent.) Barnhart]. Researchers have attributed other species of the Asteraceae family (i.e., annual ragweed [*Ambrosia artemisiifolia* L.] and common sunflower [*Helianthus annuus* L.]) to have allelopathic properties (Azania et al., 2003; Shetty et al., 2007). High-yielding legume species can compete directly for water, sunlight, and essential nutrients causing a shift in the composition of the mixture and thus reducing yield (Blanchet et al., 1995; George et al., 1995). Jones et al. (1988) reported alfalfa [*Medicago sativa* L.] and birdsfoot trefoil [*Lotus corniculatus* L.] dominated stands when planted in binary mixtures with reed canarygrass [*Phalaris arundinacea* L.] in Iowa. When intercropping legumes with switchgrass, legume growth habits can adversely affect the growth of switchgrass (George et al., 1995; Marten, 1989). Posler et al. (1993) found binary grass-legume mixtures of switchgrass-cicer milkvetch [*Astragalus cicer* L.] and switchgrass-catclaw sensitive brier [*Mimosa nuttallii* (DC. ex Britton & Rose) B.L. Turner formerly known as *Schrankia nuttallii* (DC. ex Britton & Rose) Standl.] grown in Kansas had a reduction in switchgrass percentage due to the prostrate growth habit and thick canopy cover of the legumes.

To further investigate the impact of companion crops on NWSG pastures, the research reported in the following chapters focused on evaluating how interseeding CSA grass and legume species, as well as warm-season forbs, can affect established NWSG pastures. In Chapter 1, the objective was to assess the impact of three cool-season annual seeding strategies and two warm-season N fertilization rates over three years on established switchgrass and big bluestem/indiangrass pastures. Specifically, the research evaluated NWSG plant density, switchgrass tiller density, and NWSG hay yield as affected by interseeding a cereal rye monoculture, a polyculture of cereal rye, 'Purple Top' turnip [*Brassica septiceps* (L.H. Bailey) L.H. Bailey], 'Trophy' rape [*Brassica napus* L.], 'Frosty' berseem clover [*Trifolium alexandrinum* L.], and 'Dixie' crimson clover, or a fallow control and applying 0 or 67 kg N ha^{-1} during NWSG growth. In Chapter 2, the objective was to evaluate whether within-season rest from grazing is needed for sustainability of interseeded forbs within native pasture. Two NWSG grazing studies were conducted to assess the persistence of native forbs when an 11-species native forb blend was sown into established switchgrass and big bluestem/indiangrass pastures. Specifically, the research evaluated forb plant density per species, NWSG tiller density, forage nutritive value, and forb flowering percentage of these polycultures as affected by within-season rest treatments implemented throughout the grazing season (May-August).

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CHAPTER I

INTERSEEDING WINTER ANNUALS INTO NATIVE WARM-SEASON GRASS PASTURES

ABSTRACT

Native warm-season grass (NWSG) pastures could benefit from the incorporation of cool-season annual (CSA) species by potentially extending the grazing season, reducing supplemental feed costs, suppressing weeds, and increasing herbage production and overall forage quality. Therefore, two NWSG grazing experiments were conducted in Spring Hill, TN, from 2018-2020, to assess three CSA seeding options (cereal rye [*Secale cereal* L.] monoculture, a polyculture of cereal rye, 'Purple Top' turnip [*Brassica septiceps* (L.H. Bailey) L.H. Bailey], 'Trophy' rape [*Brassica napus* L.], 'Frosty' berseem clover [*Trifolium alexandrinum* L.], and 'Dixie' crimson clover [*Trifolium incarnatum* L.], or a fallow control) and two warm-season N rates (0 or 67 kg N ha^{-1}) on established switchgrass [SG; *Panicum virgatum* L.] and big bluestem/indiangrass [BBIG; *Andropogon gerardii* Vitman and *Sorghastrum nutans* (L.) Nash] pastures. Treatments were randomly assigned in a strip plot arrangement under an unbalanced incomplete block design to eight SG and six BBIG pastures. Switchgrass and BBIG plant density were not influenced by CSA seeding but varied by Year ($P < 0.001$). Plant density for BBIG was lower in 2020 than 2018 (23.6 vs. 29.1 plants m^{-2}, respectively), whereas SG plant density was similar for 2018 and 2020 (15.0 and 14.0 plants m^{-2}, respectively). Hay yield for both SG and BBIG varied among harvests (July 2019, July 2020, and September 2020; $P < 0.001$) but not by CSA seeding. Because CSA did not affect plant density or hay yield over the course of these experiments, incorporating CSA may be a viable option for enhancing grazing opportunities and forage production during the dormant period of NWSG.

INTRODUCTION

Cool-season annuals (CSA) have been utilized in agricultural production for centuries. However, literature evaluating grazing and forage production of CSA grasses, forbs, legumes, and mixtures planted in established native warm-season grasses (NWSG) is lacking. Past research utilizing this approach has been fairly limited to one NWSG, switchgrass [SG; *Panicum virgatum* L.] (Bow, Muir, Weindorf, & Butler, 2008; Ashworth et al., 2015; Keyser et al., 2016a; Watcharaanantapong, Keyser, McIntosh, & Griffith, 2020) while predominantly focusing on a non-native, sod-forming, perennial warm-season grass, bermudagrass [*Cynodon dactylon* L. Pers.] (Hoveland, Anthony, McGuire, & Starling, 1978, 1997; Beck et al., 2007).

Including CSA grasses (e.g., cereal rye [*Secale cereal* L.] and wheat [*Triticum aestivum* L.]) or legumes (e.g., crimson clover [*Trifolium incarnatum* L.] and arrowleaf clover [*Trifolium vesiculosum* Savi]) into warm-season pasture can increase the number of grazing days per season (Hoveland et al., 1977) and lower production costs by reducing the need for supplemental feedstuffs. Hoveland et al., (1978) noted that sowing annual ryegrass [*Lolium perenne* L. subsp. *multiflorum (*Lam.) Husnot] into bermudagrass sod in Alabama extended the number of grazing days ha^{-1} by 62 (from 178 without the annual to 240 days with interseeded annuals) thus allowing for grazing more than 65% of the calendar year. Beck et al. (2007), working in Arkansas, also found increased average daily gain (ADG), animal grazing days ha^{-1}, and body weight gain ha^{-1} of growing calves when interseeding annual ryegrass and small grains into bermudagrass as opposed to small grains alone. In Tennessee, Fribourg and Overton (1973) noted that bermudagrass pastures overseeded with cereal rye, oats [*Avena sativa* L.], wheat, and ryegrass could have allowed for grazing from mid-February through September.

With warm-season pastures, intercropping cool-season legumes has multiple benefits. By planting cool-season legumes in early fall, winter and spring growth can stabilize and protect the soil (Keeling et al., 1996) while WSG are dormant. As is the case with CSA grasses, incorporating cool-season legumes into WSG can extend the overall grazing season (Springer, 1997) and increase herbage production (George et al., 1995). Adding legumes to a forage production system increases CP, forage intake, and overall animal performance (Blaser et al., 1956; Vallis, 1976; Van Soest, 1976; Marten, 1985). Ashworth et al. (2015), working in Tennessee, reported that red clover [*Trifolium pratense* L.] and ladino clover [*Trifolium repens* L.] as well as a native warm-season legume, partridge pea [*Chamaecrista fasciculata* (Michx.) Greene], can be established in SG swards without being reseeded for ≥3 years. Bow et al. (2008) reported that SG-arrowleaf clover and SG-common vetch [*Vicia sativa* L.] plots grown without compost in central Texas produced an average of 60% greater herbage than SG monocultures in the second year of the study.

Not all well-adapted legume species are suitable for use in binary mixtures or polycultures with grasses; high yielding legume species, for instance, can compete directly with grasses and thus reduce yield (Blanchet et al., 1995). Differences in growth habits, such as plant stature (erect versus prostrate; Ashworth et al., 2015) and days to maturity, influence the compatibility of binary mixtures. One species, either the grass or the legume, tends to outcompete the other for water, sunlight, and essential nutrients causing a shift in the composition of the mixture (George et al., 1995). Jung et al. (1985)

failed to establish legumes when broadcasting seed in thick stands (>75%) of SG and BB in March in Pennsylvania.

On the contrary, when intercropping legumes, legume growth habits can potentially adversely affect the growth of SG (Marten, 1989; George et al., 1995; Keyser et al., 2016b). Cool-season legume growth that extends into the early portion of SG growth could cause yield and stand reductions. Taylor and Jones (1983) found that following the second year of their study in Kentucky, red clover intercropped with SG began to dominate the polyculture. However, N fertilization may help offset these negative effects by increasing grass growth. Researchers fertilized SG with 151 (Obour, Harmoney, & Holman, 2017) and 168 kg N ha^{-1} (Muir, Sanderson, Ocumpaugh, Jones, & Reed, 2001) in Texas and 120 kg N ha^{-1} in Iowa and Nebraska (Vogel, Brejda, Walters, & Buxton, 2002) in order to maximize yield. However, fertilizing SG with 65 (Haque, Epplin, & Taliaferro, 2009) and 69 kg N ha^{-1} (Aravindhakshan, Epplin, & Taliaferro, 2011) maximized both yield and profit in Oklahoma. Currently, 67 kg N ha^{-1} is the recommended rate for SG forage production (Keyser et al., 2011a; Popp et al., 2018).

Interseeded CSA have the potential to enhance NWSG pastures. Furthermore, because CSA small grains exhibit allelopathic properties (Liu & Lovett, 1993; Kato-Noguchi et al., 1994; Fomsgaard, 2006), interseeding them into established NWSG could organically aid in weed control as opposed to using herbicides for the same result (Keyser et al., 2016b). Therefore, we conducted two NWSG field experiments to assess the impact of three cool-season annual seeding strategies and two warm-season N fertilization rates over three years on established SG (experiment 1) and big bluestem/indiangrass (BBIG; experiment 2) pastures. Specifically, we evaluated NWSG plant density, SG tiller density, and NWSG hay yield as affected by interseeding a cool-season annual monoculture of cereal rye, a polyculture of cereal rye, 'Purple Top' turnip [*Brassica septiceps* (L.H. Bailey) L.H. Bailey], 'Trophy' rape [*Brassica napus* L.], 'Frosty' berseem clover [*Trifolium alexandrinum* L.], and 'Dixie' crimson clover, or a non-planted control and applying 0 or 67 kg N ha^{-1}. We hypothesized that by incorporating a CSA, grazing days ha^{-1} would increase for each year, but NWSG plant density, SG tiller density, and thus hay yield would decrease over time.

MATERIALS AND METHODS

Site Description

Two NWSG field experiments (one evaluating a BBIG blend and one SG) were conducted at the University of Tennessee – Middle Tennessee AgResearch and Education Center (MTREC; 35°42'29.99" N, 86°56'38.46" W) in Spring Hill, TN, from 2018-2020. The soil consisted of a well-drained, Maury silt loam (fine, mixed, active, mesic Typic Paleudalfs) and a well-drained, Armour silt loam (fine-silty, mixed, active, thermic Ultic Hapludalfs) (48 and 15%, respectively) (NRCS Web Soil Survey, 2021). An initial soil test was conducted from 0-15-cm depth to assess P, K, and lime requirements based on soil test results (Mehlich 1) for "Medium" levels (University of Tennessee Soil, Plant and Pest Center, Nashville, TN) and pH > 5.0 (Keyser et al., 2011a; Keyser, Harper, Bates, Waller, & Holcomb, 2011b). Mean SG pasture soil pH was 5.7 with 469 kg ha^{-1} P (Very High) and 115 kg ha^{-1} K (Medium). Mean BBIG pasture soil pH was 5.9 with 262 kg ha^{-1} P (Very High) and 187 kg ha^{-1} K (High). Mean monthly air temperature and precipitation were collected at a weather station located on MTREC each year and compared to 30-year means (NOAA, 2021).

Experimental Design

Two treatments were randomly assigned in a strip plot arrangement under an unbalanced incomplete block design to eight SG [cv, 'Alamo'] and six BBIG [a 2:1 blend (based on seed mass) of BB and IG ecotypes collected in Kentucky that are commercially available (Roundstone Native Seed, Upton, KY)] 1.2-ha pastures (blocks) (Figure 1.1). Pastures were originally established in 2008 with native grass species randomly assigned to each pasture as part of a previous experiment. The first treatment was two planted CSA and a non-planted control. Cool-season annual treatments were a cereal rye monoculture, CSA polyculture (cereal rye, 'Purple Top' turnip, 'Trophy' rape, 'Frosty' berseem clover, and 'Dixie' crimson clover), and a fallow control. Each pasture received only one CSA treatment paired with the control. The second treatment, warm-season N fertilization, was superimposed across half of each pasture such that each 1.2-ha pasture included a factorial combination of the two treatments. Warm-season N fertilization rates were 0 kg N ha^{-1} (control) and 67 kg N ha^{-1} (Keyser et al., 2011a; Popp et al., 2018) in the form of urea [$CO(NH_2)_2$] with a urease inhibitor. Nitrogen treatments were applied on 23 May 2019 and 14 May 2020. Each block was replicated four times for SG and three times for BBIG.

Weed control needs for pastures were assessed prior to study commencement. An application of glyphosate {N-[phosphonomethyl] glycine, isopropyl-amine salt; 41%} at 1.89 L product ha^{-1} was applied to all SG pastures in April 2017 due to excessive broomsedge [*Andropogon virginicus* L.] infestations. No other herbicides were applied during the remainder of the experiments.

Prior to planting CSA, SG and BBIG pastures were clipped with a John Deere HX15 flew-wing rotary cutter (Deere & Company, Moline, IL) to a 20-cm stubble height. Cool-season annual treatments were no-till drilled into SG and BBIG pastures in September 2018 and 2019 using a 15-row Great Plains® Model 1006NT (Great Plains Manufacturing, Inc., Salina, KS) no-till drill with 19.1-cm row spacing. Pure live seeding (PLS) rates ha^{-1} were: 125.4 kg cereal rye (monoculture) and 94.1 kg cereal rye, 1.1 kg turnip, 0.6 kg rape, 4.5 kg 'Frosty' berseem clover, and 5.6 kg 'Dixie' crimson clover (polyculture). Cool-season annuals received two applications (November and February) of 33.6 kg N ha^{-1} in the form of urea [$CO(NH_2)_2$] with a urease inhibitor and 39.2 kg K_2O ha^{-1} prior to each CSA grazing season.

Animal Management and Measurements

Grazing only occurred during the cool-season because management objectives were focused on warm-season grass hay production. Black Angus/Angus-cross cow-calf pairs were used for cool-season grazing. Grazing commenced on 2 April 2019 and 9 March 2020 based on CSA forage availability. Grazing ended on 2 May 2019 and 15 April 2020. Grazing days ha^{-1} were recorded for both studies based on CSA treatments (Table 1.1). In 2019, five cow-calf pairs per CSA treatment rotationally grazed pastures for both experiments. In 2020, two cow-calf pairs (n = 16 for SG and n = 12 for BBIG) grazed each pasture for the duration of the CSA grazing period. Two additional pairs were added to one cereal rye treatment pasture due to forage availability. Animals had access to the entire pasture for grazing and *ad libitum* availability to mineral, water, and shade. Mean initial body weight (IBW) (±SE) of cow-calf pairs was 547.6 ± 11.5 and 549.1 ± 8.6 kg for cows in 2019 and 2020, respectively, and 33.2 ± 1.4 and 72.2 ± 4.5 kg for calves in 2019 and 2020, respectively. Animal care adhered to UT-Institutional Animal Care and Use protocols No. 2258-0417 and No. 2258-0320.

Pasture Management and Measurements

Prior to CSA grazing, CSA stands were characterized by CSA plant cover (Table 1.2), CSA plant density (Table 1.3), and nutritive value (Table 1.4). Plant cover was measured using a 0.20-m^2 frequency grid (Vogel & Master, 2001) at 20 random locations within each CSA treatment in each pasture. Plant density was assessed annually prior to initiation of grazing by counting plants within the same 20 random 0.20 m^2 locations. Counts were taken ≥4 m from the boundary of another treatment, fence, shade, or other heavily traveled area. Concurrent with plant counts, CSA forage samples were collected at a 5-cm stubble height for forage nutritive value analysis using near-infrared reflectance spectroscopy (NIRS).

Annually, standing NWSG biomass was harvested for hay yield to a 20-cm stubble height. Hay harvesting equipment included: John Deere 4-basket fold-up hay tedder, and 557 1.5 m x 1.5 m mega wide baler (Deere & Company, Moline, IL), Kuhn FC 313 RTG disc mower conditioner (Kuhn North America, Inc., Brodhead, WI), and Durabilt HC-3110 10-wheel hay rake (Durabilt Industries, LLC., Pocahontas, AR). Harvests were conducted consistently on all experimental units to avoid biasing N and CSA treatment effects on stand vigor. In 2019, a warm-season harvest occurred on 22

July for both SG and BBIG. Regrowth of NWSG following initial harvest in 2019 was minimal enough that it did not warrant hay harvest in September prior to CSA planting. In 2020, harvests occurred on 13 July and 6 July for SG and BBIG, respectively, and again on 4 September for both. Number and mass (Tru-Test™ Scale EziWeigh7, Mineral Wells, TX) of a subset (n = 10 per NWSG) harvested bales were recorded. Following the July 2019 and 2020 hay harvests, hay core samples (n = 8) were collected from eight bales within each pasture using a 45.7-cm (1.9-cm diameter) Model 2002 Colorado Hay Probe (UDY Corporation, Fort Collins, CO).

Native WSG stands were assessed annually (September-October) by counting plants on a 0.25 m^2 basis at 10 random locations within each experimental unit (SG n = 32; BBIG n = 24). Adjacent grass bunches separated by ≥7.6 cm at ground level were considered individual plants. Plants were tallied if ≥50% of the crown was within the 0.25 m^2 quadrat or if ≥50% of the 0.25 m^2 quadrat was covered by the plant. For SG, tillers $plant^{-1}$ were counted for the SG plant closest to the upper right corner of the 0.25 m^2 quadrat and categorized as follows: 1 (0-25 tillers), 2 (26-50 tillers), 3 (51-75 tillers), 4 (76-100 tillers), 5 (101-125 tillers), and 6 (>126 tillers).

Nutritive Value Analysis

Following CSA sample and NWSG hay core sample collections, collected samples were dried at 55°C in a forced-air oven (Wisconsin Oven Corporation, East Troy, WI) for at least 72 hours. Samples were then ground to pass a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) for forage nutritive value analysis. Nutritive value estimates of CP, acid detergent fiber (ADF), amylase neutral detergent fiber (aNDF), and *in vitro* true dry matter digestibility following a 48-hour incubation (IVTDMD48h) were predicted via NIRS using a SpectraStar 2600 XT-R using UScan software (Unity Scientific, Milford, MA). The 2018 Grass Hay and Mixed Hay calibrations provided by the NIRS Forage and Feed Consortium (NIRSC, Hillsboro, WI) were standardized and checked for accuracy by the Global H statistical test comparing the scanned samples against the calibration ($H < 3.0$) and are reported accordingly (Murray and Cowe, 2004).

Statistical Analysis

Native WSG plant density, tillers per plant (SG only), and NWSG hay yield and nutritive value were analyzed under an analysis of variance (ANOVA) model using PROC MIXED in SAS® software, Version 9.4 (SAS Institute, Cary, NC, 2013) for significant differences at $\alpha = 0.05$ among fixed effects and their interactions. For plant density and tillers per plant, fixed effects were CSA (cereal rye monoculture, CSA polyculture, or fallow control), warm-season N rates (0 or 67.2 kg N ha^{-1}), and year (2018, 2019, or 2020). Year was treated as a repeated factor. For NWSG hay yield, CSA and harvest (July 2019, July 2020, or September 2020) were fixed effects. Harvest was treated as a repeated measure. For NWSG hay nutritive value (CP, ADF, aNDF, and IVTDMD48h), CSA and year (July 2019 or July 2020) were fixed effects with year treated as a repeated measure. Pasture was entered as a random effect in all models. Mean separations were conducted using Tukey's honestly significant difference for all significant response variables.

Switchgrass tiller categories were analyzed under a Chi-Square test using PROC FREQ in SAS® software, Version 9.4 (SAS Institute, Cary, NC, 2013) for significant differences ($\alpha = 0.05$) among CSA treatments and warm-season N rates pooled across years (2018, 2019, and 2020). Number of SG plants with ≤25 tillers were compared to SG plants with ≥26 tillers.

RESULTS AND DISCUSSION

Environmental Conditions

From September 2018-September 2020, mean monthly air temperatures were similar to or above (15 of 25 months) 30-year means with NWSG growing season (April through September) mean monthly air temperatures following suit (7 of 13 months) (Figure 1.2a). Monthly precipitation followed the same pattern with 14 out of 25 months greater than 30-year means (Figure 1.2b). Also, just under half (6 of 13) of the months during the NWSG growing season had greater monthly precipitation. September 2018 and 2019 had greater mean monthly temperature than 30-year mean, while 2018 had abnormally greater (108%) precipitation than 30-year mean. In contrast, September 2019 was uncharacteristically dry compared to 30-year mean (98% lower) with October having greater (88%) than average rainfall. February 2019 and 2020 were wetter than average (107 and 82%, respectively) followed by greater than 30-year mean precipitation in March 2020 (35%), too. However, the first three months (April-June) of the NWSG growing season in 2020 had abnormally low precipitation in May and June while June 2019 met the 30-year mean (NOAA, 2021).

Density

In both studies, plant density was not influenced by CSA or any CSA interaction (Table 1.5). Cool-season annual plant density averaged 483.3 plants m^{-2} across both NWSG, CSA treatments, and years (Table 1.3). Complete graze-out of CSA in both years could have aided in the lack of any differences among CSA treatments. Other researchers have also observed similar results when interseeding CSA into NWSG. In Tennessee, Watcharaanantapong and others (2020) overseeded SG with cereal rye, wheat, and annual ryegrass and found no difference in SG stand vigor after two consecutive years of planting and harvesting CSA. In Alabama, Mason et al. (2019), working with eastern gamagrass [EG; *Tripsacum dactyloides* (L.) L.], another native warm-season bunchgrass, found comparable results. They reported that overseeding EG with cereal rye or cereal rye-red clover mixtures did not affect EG persistence after two years.

Year was the overarching effect on SG ($P < 0.001$) and BBIG ($P < 0.001$) plant density. For SG, plant density increased from 2018 (15.0 plants m^{-2}) to 2019 (18.2 plants m^{-2}) and then decreased in 2020 (14.0 plants m^{-2}) to a density similar to 2018. For BBIG, plant density increased from the initial count in 2018 (29.1 plants m^{-2}) to 2019 (43.9 plants m^{-2}) and then decreased in 2020 (23.6 plants m^{-2}). Weather conditions may have played a role in this decrease in plant density since temperatures during green-up were lower in 2020 than 2019. However, total monthly precipitation during the NWSG growing season was greater for all months, except June, in 2020 than in 2019. Even though NWSG plant densities were greatest in 2019 and returned to similar or below 2018 densities in 2020, stands were well above fully stocked densities ($\geq$11 plants m^{-2};

Keyser, Harper, Bates, Waller, & Holcomb, 2011c) in all years. The elevated densities in 2019 may also be attributed to sampling error in counting individual plants. Conducting plant counts within dense (i.e., 14-43 plants m^{-2}) 10-year-old NWSG stands underscores the difficulty of counting individual plants within such robust stands.

Although both SG and BBIG had the greatest plant density following the first year of CSA grazing, the difference in NWSG plant density following the first and second year of grazing could have resulted from the timing of grazing instead of the CSA treatment. Grazing occurred a month earlier in 2020 (March-April) than 2019 (April-May) due to a period of drought that slowed CSA growth in 2019 whereas no drought was recorded in 2020. Grazing earlier would potentially allow for greater regrowth of grazed CSA prior to NWSG growth. Also, CSA plant density was numerically greater for CSA treatments in 2020 than in 2019 (except for the SG CSA polyculture) (Table 1.3). Increasing competition for resources with larger established CSA plants could inhibit NWSG green-up. Fribourg and Overton (1973) reported reduced bermudagrass yields when interseeding annual ryegrass and attributed this reduction to overlapping growth where the CSA impeded the warm-season perennial's initial growth following dormancy. However, in the current studies, NWSG plant density was no different among the CSA plantings and the non-planted control. Thus, the change in grazing management from year-to-year may have influenced mean plant density. Continually stocking each pasture for the duration of the 2020 CSA grazing period, as opposed to rotational stocking all pastures in 2019, may have allowed cattle access to NWSG at green-up in all pastures in 2020 as opposed to a single pasture per species in 2019. Furthermore, grazing prior to NWSG reaching 38-cm tall could potentially weaken stands (Keyser, Bates, Waller, Harper, & Holcomb, 2011d).

Timing and frequency of hay harvest could have influenced NWSG plant density. Both NWSG were harvested on 22 July which is after SG, BB, and IG have begun to reach reproductive stage. Branson (1953) found that declines in SG stand density and plant vigor were attributed to a high ratio of reproductive to vegetative stems at harvest. Another possibility for the decline in plant density in 2020 could be attributed to the two harvests in 2020. Beaty and Powell (1976) noted that SG plant and crown survival declined when harvested two or more times per year.

For SG tillers plant^{-1}, only warm-season N treatment was significant (P = 0.048). Experimental units fertilized with 67 kg N ha^{-1} had greater mean number of tillers plant^{-1} than 0 kg N ha^{-1} (1.83 v. 1.63, respectively). Switchgrass tiller categories were also not influenced by CSA (P = 0.241, x^2 = 2.84) but by warm-season N treatment (P = 0.034, x^2 = 4.52). Furthermore, there was a greater percentage of fertilized SG plants (43.4%) having ≥26 tillers as compared to non-fertilized plants (36.6%). Because this was not the case for SG plant density, it seems apparent N fertilization increased individual SG plant vigor without increasing the total number of plants.

Within the BBIG experiment, BB and IG accounted for 52.3 and 47.7%, respectively, of the BBIG stands pooled across all years, pastures, and treatments. Plant density for BBIG was influenced by warm-season N treatment (P < 0.001) but not by any interactions between fixed effects (Table 1.5). For warm-season N treatment, 0 kg N ha^{-1} had greater mean plant density than 67 kg N ha^{-1} (34.9 and 29.6 plants m^{-2}, respectively), which is contrary to the results for SG tiller plant^{-1}. Because BB and IG do not mature as early as SG, the May N fertilizer application may have added to increased weed density thus outcompeting BB and IG. However, increased weed pressure was not visually apparent during NWSG counts. Alternatively, the decrease in plant density could have been due to growth and/or expansion of individual plants resulting from N fertilization. McKendrick, Owensby, and Hyde (1975) reported that each vegetatively reproductive tiller of BB produced approximately two tillers the following year. Based on the current sampling protocol, the larger expanding plants could have encompassed the area (7.6 cm) between individual plants thus causing difficulty in differentiating between one or multiple plants in close proximity within a 10-year-old NWSG stand.

Hay Yield, Nutritive Value, and Grazing Days

Hay yield followed the same trajectory as plant density. Hay yield for both SG and BBIG was only influenced by Harvest ($P < 0.001$; Table 1.6). For SG, the July 2019 (6.5 Mg ha^{-1}) harvest had the greatest hay yield, which was representative of the greatest plant density, while July 2020 (4.3 Mg ha^{-1}) and September 2020 (4.8 Mg ha^{-1}) were similar to one another. For BBIG, July 2020 (5.2 Mg ha^{-1}) produced the lowest hay yield while July 2019 (7.1 Mg ha^{-1}) was similar to September 2020 (7.0 Mg ha^{-1}). The decline in hay yield could have been due to mean monthly temperature during the green-up period. Temperatures in April, May, and June of 2019 were greater than those in 2020, allowing for dormancy break of NWSG to occur earlier in the year. Furthermore, the decline in hay yield, and potentially plant density, from July 2019 to July 2020 could have been influenced by the July 2019 harvest. Dwyer et al. (1963) and Newell and Keim (1947) reported a decline in SG yield the year following the first harvesting year.

When analyzing hay nutritive values, the CSA x Year interaction only influenced aNDF for SG (P = 0.046; Table 1.7). The CSA polyculture was greatest for aNDF (748. 2 g kg^{-1}) in 2020 while all other interactions did not differ. Crude protein differed by Year for SG and BBIG (P = 0.007 and P = 0.016, respectively). Both SG and BBIG had greater CP in 2020 (82.5 and 79.2 g kg^{-1}, respectively) than 2019 (74.4 and 70.0 g kg^{-1}, respectively). However, this year effect could be attributed to plant maturity since both NWSG were harvested earlier in 2020 than in 2019. For SG, the cereal rye monoculture pastures had lower ADF (414.7 g kg^{-1}) than CSA polyculture (431.8 g kg^{-1}) pastures (P = 0.049). For BBIG, Year affected ADF content with lower fiber in 2020 (426.7 g kg^{-1}) than 2019 (442.5 g kg^{-1}), which again could be associated with reduced plant maturity in 2020 than 2019.

Although, SG and BBIG plant density, SG tiller density, and hay yield did not differ among CSA treatments, planting CSA added forage production during the dormant season prior to NWSG growth. Across CSA treatments and grazing years, SG averaged 48.5 grazing days ha^{-1} while BBIG averaged 51.7 grazing days ha^{-1} for cow-calf pairs. This high-quality forage had average CP, ADF, aNDF, and IVTDMD48H values of 198.2, 198.7, 417.6, and 908.6 g kg^{-1}, respectively, across both NWSG forages, CSA treatments, and years. Mason et al. (2019) reported mean CP values for CSA in February slightly above (206 g kg^{-1}) those in the current studies while March/April (110 g kg^{-1}) were well below. Their values were averaged across cereal rye monoculture and a cereal rye-red clover mixture.

CONCLUSIONS

Based on these results, it appears CSA can be interseeded into SG and BBIG pastures without any negative impact on NWSG. The decline in plant density in the third year of the study may have been impacted by multiple factors, such as differing weather conditions from year-to-year, CSA plant density, and management practices (timing of CSA grazing, NWSG hay harvest timing and frequency, etc.). However, the most plausible explanation may be the difficulty of separating individual plants from one another within the mature, robust NWSG stands in this study. In regard to applying N fertilizer during the warm season, no consistent response was observed. Planting CSA with NWSG increased grazing days ha^{-1} and provided high quality forage during NWSG dormancy. Based on nutritive values, planting a CSA polyculture that includes legumes would have greater potential to increase animal performance than a cereal rye monoculture.

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APPENDIX I

Table 1.1. Grazing days ha^{-1} for cow-calf pairs grazing interseeded cereal rye monoculture (RYE) and cereal rye, crimson clover, berseem clover, turnip, and rape polyculture (Poly) within switchgrass and big bluestem/indiangrass pastures, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN.

	Switchgrass		Big bluestem/Indiangrass	
Year	RYE	Poly	RYE	Poly
	---------------------------d ha^{-1}-----------------------------			
2019	31.3	31.3	41.7	41.7
2020	69.6	61.7	61.7	61.7

Table 1.2. Plant cover (% m^{-2}) of cool-season annual treatments of interseeded cereal rye and polyculture (cereal rye, turnip, rape, berseem clover, and crimson clover) within switchgrass and big bluestem/indiangrass pastures prior to grazing, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN.

		Switchgrass		Big bluestem/Indiangrass	
Cool-season Annual		2019	2020	2019	2020
		-------------------------% m^{-2}-------------------------			
Monoculture					
	Cereal Rye	84.6	74.9	65.7	83.8
Polyculture					
	Cereal Rye	78.8	66.0	72.9	70.7
	Turnip	<0.1	0.0	<0.1	0.0
	Rape	0.0	0.0	<0.1	<0.1
	Berseem clover	6.1	2.7	12.9	5.7
	Crimson clover	31.2	20.0	78.3	39.1

Table 1.3. Cool-season annual plant density (plants m^{-2}) of interseeded cereal rye and polyculture (cereal rye, turnip, rape, berseem clover, and crimson clover) within switchgrass and big bluestem/indiangrass pastures prior to grazing, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN.

		Switchgrass		Big bluestem/Indiangrass	
Cool-season Annual		2019	2020	2019	2020
		------------------------plants m^{-2}------------------------			
Monculture					
	Cereal Rye	498.3	646.1	348.1	713.1
Polyculture					
	Cereal Rye	419.1	333.6	316.6	396.9
	Turnip	0.1	0.0	0.1	0.0
	Rape	0.0	0.0	0.2	0.2
	Berseem clover	7.3	3.4	10.7	6.4
	Crimson clover	26.6	24.6	63.2	55.0

Table 1.4. Mean crude protein (CP), acid detergent fiber (ADF), amylase neutral detergent fiber (aNDF), and *in vitro* true dry matter digestibility 48 hours (IVTDMD48H) of interseeded cereal rye (RYE) and polyculture (cereal rye, turnip, rape, berseem clover, and crimson clover) within switchgrass and big bluestem/indiangrass pastures prior to grazing, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN.

		Switchgrass				Big bluestem/Indiangrass			
Year	Cool-season Annual	CP	ADF	aNDF	IVTDMD48	CP	ADF	aNDF	IVTDMD48
		--g kg^{-1}--							
2019	RYE	123.2	254.2	506.6	862.9	140.9	202.4	422.2	885.8
2019	Polyculture	147.2	236.2	456.7	891.9	167.8	205.4	391.2	887.3
2020	RYE	243.1	171.7	393.4	926.8	240.9	181.4	397.4	920.7
2020	Polyculture	264.6	170.7	395.4	948.6	258.1	168.1	377.8	945.2

Table 1.5. Mixed-effects ANOVA model for switchgrass and big bluestem/indiangrass plant density (plants m^{-2}) and switchgrass tillers ($plant^{-1}$) in native pasture grazing experiments, 2018-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN.

	Switchgrass				Big bluestem/Indiangrass	
	Plant		Tiller		Plant	
Effect	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
CSA†	0.36	0.709	3.20	0.090	3.26	0.147
N	0.83	0.389	5.85	**0.048**	17.20	**<0.001**
Year	14.13	**<0.001**	0.38	0.689	56.51	**<0.001**
CSA x N	0.31	0.737	1.61	0.228	0.86	0.442
CSA x Year	1.67	0.173	0.39	0.816	2.13	0.099
N x Year	1.69	0.195	0.06	0.943	1.94	0.160
CSA x N x Year	0.70	0.594	0.62	0.654	0.61	0.655

†CSA = cool-season annual (cereal rye monoculture or cereal rye, turnip, rape, berseem clover, and crimson clover polyculture); N = warm-season N treatment (0 or 60 kg ha^{-1}); Year = 2018, 2019, or 2020

Table 1.6. Mixed-effects ANOVA model for hay yield (Mg ha^{-1}) by cool-season annual (CSA) pasture for switchgrass and big bluestem/indiangrass pastures in native pasture grazing experiment, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN.

	Switchgrass		Big bluestem/Indiangrass	
Effect	*F* value	*P* > *F*	*F* value	*P* > *F*
CSA†	0.37	0.564	2.02	0.234
Harvest	39.52	**<0.001**	29.26	**<0.001**
CSA x Harvest	2.01	0.200	0.43	0.666

†CSA = cool-season annual (cereal rye monoculture or cereal rye, turnip, rape, berseem clover, and crimson clover polyculture); Harvest = warm-season hay yield (July 2019, July 2020, or September 2020)

Table 1.7. Mixed-effects ANOVA model for nutritive value parameters for hay samples by cool-season annual (CSA) pasture for switchgrass and big bluestem/indiangrass pastures in native pasture grazing experiment, 2019-2020, University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN.

	Switchgrass							
	CP†		ADF		aNDF		IVTDMD48H	
Effect	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
CSA‡	0.61	0.465	6.08	**0.049**	3.34	0.118	1.27	0.302
Year	15.71	**0.007**	0.16	0.700	5.53	0.057	0.13	0.731
CSA x Year	3.72	0.102	2.54	0.162	6.30	**0.046**	3.03	0.133

	Big bluestem/Indiangrass							
	CP		ADF		aNDF		IVTDMD48H	
Effect	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
CSA‡	6.71	0.061	2.03	0.227	0.07	0.807	0.04	0.850
Year	15.87	**0.016**	14.31	**0.019**	0.49	0.521	5.46	0.080
CSA x Year	1.08	0.357	0.23	0.655	0.53	0.506	0.02	0.895

†CP = crude protein, ADF = acid detergent fiber, aNDF = amylase neutral detergent, IVTDMD48H = *in vitro* true dry matter digestibility 48 hours

‡ CSA = cool-season annual (cereal rye monoculture or cereal rye, turnip, rape, berseem clover, and crimson clover polyculture); Year = warm-season hay yield (July 2019 or July 2020)

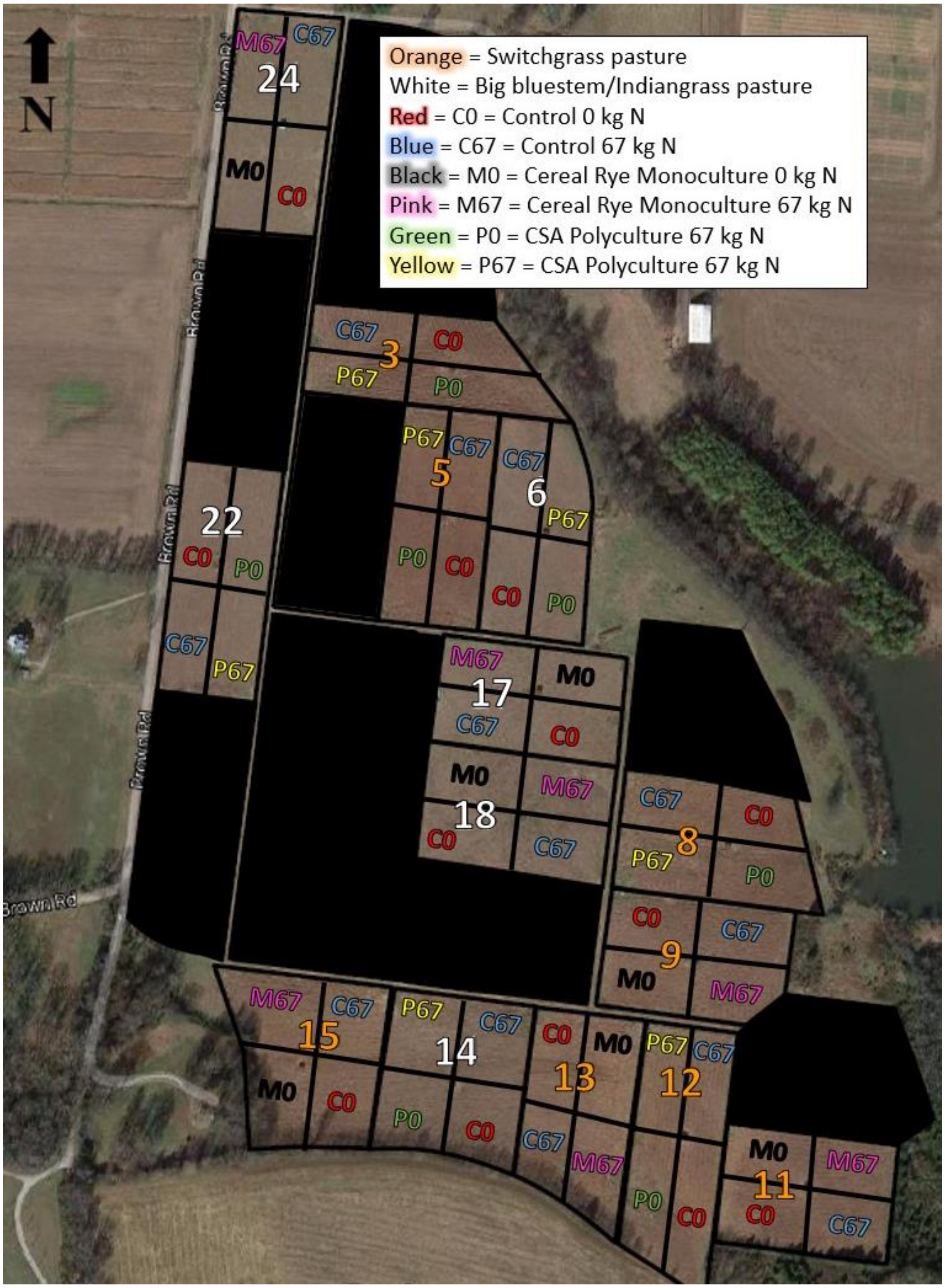

Figure. 1.1. Field layout of three cool-season annual (CSA) treatments (non-planted control, cereal rye monoculture, and CSA polyculture of cereal rye, ‘Purple Top’ turnip, ‘Trophy’ rape, ‘Frosty’ berseem clover, and ‘Dixie’ crimson clover) coupled with two warm-season N fertilization rates (0 and 67 kg N ha^{-1}) within 14, 1.2-ha pastures (eight switchgrass and six big bluestem/indiangrass mixture) at the University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN, 2018-2020.

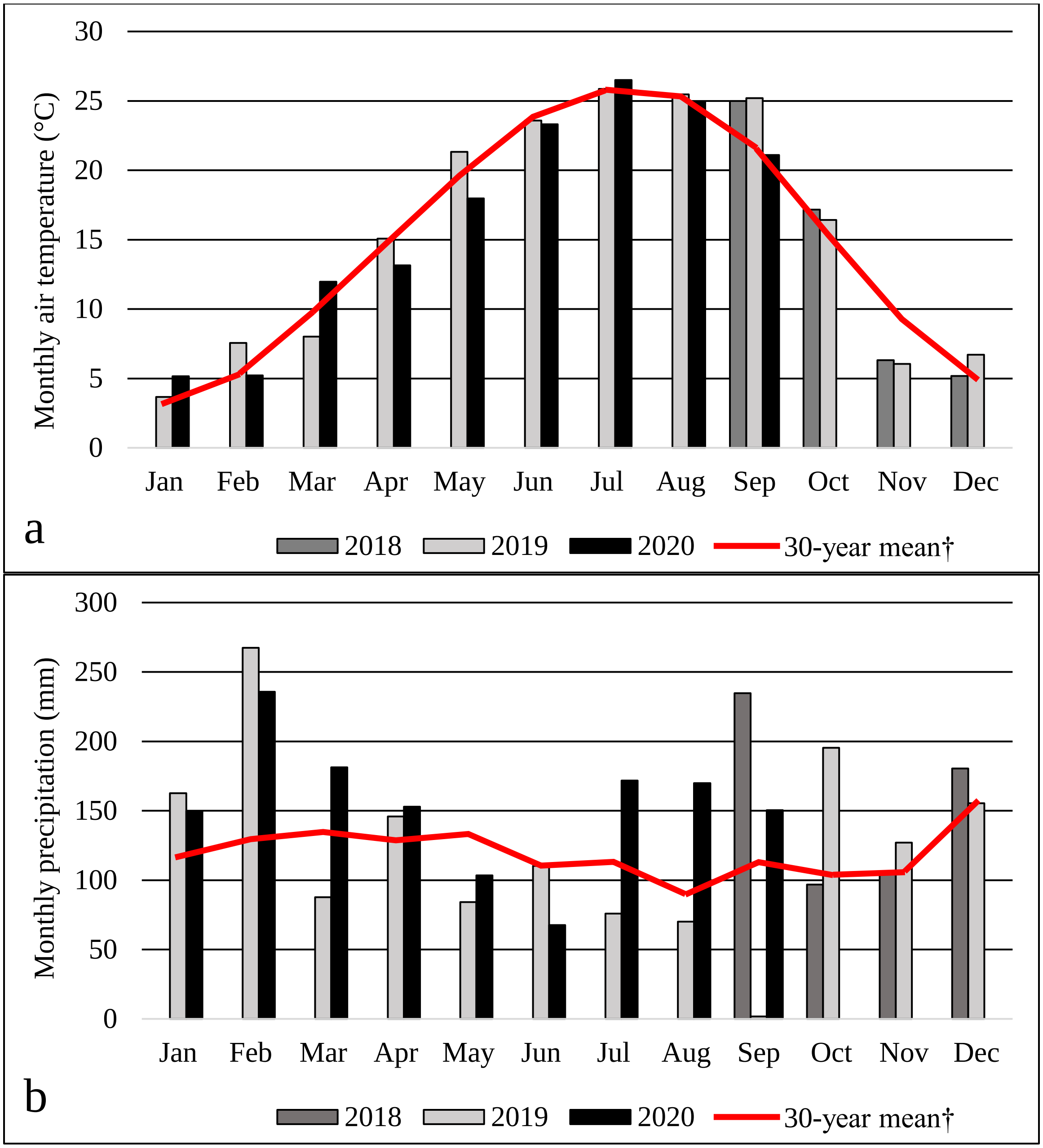

Figure. 1.2. (a) Mean monthly air temperature (°C) and 30-year mean and (b) total monthly precipitation (mm) and 30-year mean for the University of Tennessee – Middle Tennessee AgResearch and Education Center, Spring Hill, TN, 2018-2020. †Some months' data are missing in overall 30-year mean from 1991-2020.

CHAPTER II

NATIVE FORBS INTERSEEDED INTO NATIVE GRASS PASTURES PERSIST UNDER GRAZING

ABSTRACT

Incorporating native forb species within native warm-season grass (NWSG) pastures has the potential to benefit cattle, pollinators, and wildlife beyond that of NWSG monocultures. However, when grazing NWSG pastures, rotational stocking is recommended as opposed to continuous stocking. Therefore, to evaluate whether within-season rest treatments are needed for native pasture sustainability, two NWSG grazing experiments were conducted near Greeneville, TN, 2017-2020, to assess the persistence of native forbs when an 11-species native forb blend was interseeded into established switchgrass [SG; *Panicum virgatum* L.] and big bluestem/indiangrass [BBIG; *Andropogon gerardii* Vitman and *Sorghastrum nutans* (L.) Nash] pastures. Each experiment was a completely randomized design with four replicates of each within-season rest treatment (no rest, early rest, middle rest, late rest, and no graze). Within-season rest treatment was not influential for total forb plant density or NWSG tiller density thus indicating persistence of forbs may not require rotational grazing. Based on establishment and flowering during the current studies, purple prairie clover [*Dalea purpurea* Vent.] never established while Illinois bundleflower [*Desmanthus illinoensis* (Michx.) MacMill. ex B.L. Rob. & Fernald] was only observed flowering once despite having the greatest seeding rate among the forbs. Of the 11-species in the current mixture, interseeding a 6-species polyculture of black-eyed susan [*Rudbeckia hirta* L.], Dixie ticktrefoil [*Desmodium tortuosum* (Sw.) DC.], lanceleaf coreopsis [*Coreopsis lanceolata* L.], Maximilian sunflower [*Helianthus maximiliani* Schrad.], oxeye sunflower [*Heliopsis helianthoides* (L.) Sweet], and purple coneflower [*Echinacea purpurea* (L.)

Moench] could allow for plant biodiversity while offering floral resources for pollinators during the NWSG grazing season.

INTRODUCTION

Native WSG are utilized for forage and biomass production, wildlife habitat restoration, riparian buffers, roadside revegetation efforts, and controlling soil erosion. Some NWSG that have received attention as forage crops include big bluestem [BB; *Andropogon gerardii* Vitman], indiangrass [IG; *Sorghastrum nutans* (L.) Nash], little bluestem [LB; *Schizachyrium scoparium* (Michx.) Nash], and switchgrass [SG; *Panicum virgatum* L.]. Multiple researchers have evaluated NWSG for cattle grazing (Keyser et al., 2016; Backus et al., 2017; Brazil, Keyser, Bates, Saxton, & Holcomb, 2020) in the Mid-South U.S. Backus and others (2017), working with weaned steers, reported season-long average daily gains (ADG) of 0.89 and 0.68 kg d^{-1} and total gains of 335.7 and 393.8 kg ha^{-1} for a BBIG blend and SG, respectively, across two sites. Keyser et al. (2016) found greater ADG (1.25 and 1.03 kg ha^{-1} for a BBIG blend and SG, respectively) when grazing bred heifers. Brazil et al. (2020) reported an ADG between the prior researchers (0.98 kg d^{-1}) when steers continuously grazed a three-species blend of BB, IG, and LB. They also reported a total gain of 379 kg ha^{-1}.

Enhancing established NWSG pastures by incorporating native forb and legume species has the potential to increase overall sward forage quality (McGraw, Shockley, Thompson, & Roberts, 2004) and benefit cattle (Tracy & Faulkner, 2006), pollinators (Frankie et al., 2005; Tuell, Fiedler, Landis, & Isaacs, 2008), and wildlife (Harper et al., 2007) beyond that of NWSG monocultures. Posler, Lenssen, and Fine (1993) working in

Kansas reported greater crude protein (CP) concentrations in four out of five binary mixtures containing native legumes and NWSG when compared to NWSG monocultures. Springer, Aiken, and McNew (2001) reported CP values averaged across SG, BB, and IG monocultures were lower than those of SG, BB, and IG in binary mixtures with Illinois bundleflower (ILBF). Such enhancements could also qualify for financial and technical assistance under the Conservation Stewardship Program (USDA-NRCS).

When grazing NWSG pastures, rotational stocking, as opposed to continuous stocking, is recommended to help avoid overgrazing (Henning, 1993; Harper et al., 2007). However, Brazil et al. (2020) found overall mean tiller density for BB, IG, and LB increased by 14% from year three to the final year when continuously grazed in Tennessee. Also in Tennessee, Keyser et al. (2016) reported that NWSG coverage did not decrease while continuously stocking pastures of SG or BBIG interseeded with red clover. On the other hand, Bonin, Lal, and Tracy (2014) rotationally grazed native mono- and polycultures for three years by removing animals when WSG stubble height reached 16 cm and utilizing a ~30-d rest period between two grazing events per season as to not weaken stands in Virginia. They noted an increase in relative cover of sown species within monocultures and a decrease in weed species in 4-species mixtures. However, all species, including NWSG, were sown simultaneously. Hickman, Hartnett, Cochran, and Owensby (2004) reported that after six years of grazing, perennial forbs failed to be impacted by various stocking densities of cattle in the Tallgrass Prairie region of Kansas. They concluded that perennial forbs exhibited high stability across all grazing treatments. Fahnestock and Knapp (1993) determined that NWSG are more readily consumed than perennial forbs thus increasing plant abundance by decreasing competition.

Unfortunately, literature focused on grazing native forbs interseeded into established NWSG pastures is lacking. Therefore, the objective of the present studies was to evaluate whether within-season rest from grazing is needed for sustainability of interseeded forbs within native pasture, we conducted two NWSG grazing studies to assess the persistence of native forbs when an 11-species native forb blend was sown into established NWSG pastures. Specifically, we evaluated forb plant density per species, NWSG tiller density, forage nutritive value, and forb flowering percentage of these polycultures as affected by grazing treatments implemented throughout the grazing season (May-August).

MATERIALS AND METHODS

Site Description

Grazing studies evaluating two NWSG (2:1 BBIG blend and SG) forage options were conducted concurrently at the University of Tennessee Northeast Tennessee AgResearch and Education Center (NETREC; 36°06'34.6"N, 82°51'41.2"W) in Greeneville, TN, from 2017-2020. The soil at this location was a Dunmore loam (fine, kaolinitic, mesic Typic Paleudults). This site had previously been utilized as native-warm season grass pasture, SG and BBIG, established in 2008. Annual soil tests were conducted from 0-15-cm depth (Mehlich 1; University of Tennessee Soil, Plant and Pest Center, Nashville, TN) (Table 2.1). Each pasture (SG and BBIG) received 46.4 kg P_2O_5 ha^{-1} and 60.5 kg K_2O ha^{-1} in 2019. In 2020, each received 100.9 kg P_2O_5 ha^{-1} and 100.9 kg K_2O ha^{-1}. Mean monthly air temperature and precipitation were collected at a weather station located on NETREC (1.66 km from experiment locations) each year and compared to 30-year means (NOAA, 2020).

Experimental Design

Two, 1.2-ha pastures (one pasture per experiment) of established perennial NWSG (one SG and one BB/IG mixture) were interseeded with an 11-species biodiversity mixture of native forbs (Table 2.2). Five grazing treatments based on the timing of within-season rest (no rest, early rest, middle rest, late rest, and no grazing control; Figure 2.1 and Table 2.3) were arranged in a completely randomized design with four replicates (n = 20 plots) within each of the two NWSG pastures. Each experimental unit was approximately 0.05 ha with a 7.6-m wide center alley stretching the length of each pasture that allowed access to each unit as needed through the course of the season. Temporary electric fencing was used to open and close access to the 20 experimental units based on their assigned rest period.

Native WSG were existing stands established in 2008. In preparation for establishment of the forbs, pastures were burned in fall 2016, cut to a 20-cm stubble height with harvested material removed in spring 2017, and sprayed with PastureGard® HL [{triclopyr: 3,5,6-trichloro-2- pyridinyloxyacetic acid, butoxyethyl ester; 45.07%}; {fluroxypyr: [(4-amino-3,5-dichloro-6-fluoropyridin-2-yl)oxy]acetic acid, 1-methylheptyl ester; 15.56%}] at 2.34 L product ha^{-1} and 2,4-D Amine 4 [dimethylamine salt of 2,4-Dichlorophenoxyacetic acid; 47.3%] at 2.34 L product ha^{-1}. Following preparation, the native forb blend was no-till drilled on 12 June 2017 using a 9-row Great Plains® (Great Plains Manufacturing, Inc., Salina, KS) no-till drill with 19.1-cm row spacing. Annual forb/legume species (partridge pea and plains coreopsis) were reseeded on 26 March 2018 to enhance establishment due to the late initial planting date.

Animal Management and Measurements

Weaned beef steers (Angus cross) were purchased at a local stockyard (Knoxville Livestock Auction Center, Inc., Mascot, TN) for both experiments each year of the project. Steers (n = 2, n = 3, and n = 3, respectively, 2018-2020) were utilized as "testers" for each NWSG pasture based on similar weights and were randomly assigned to each experiment. Extra steers ("grazers") were used via a put-and-take grazing method to maintain target grass canopy heights of 35-40 cm for BBIG and 45-50 cm for SG (Keyser et al., 2016; Backus et al., 2017). Grazing was initiated in May (17, 16, and 28 May, respectively, 2018-2020) and ended in August (15, 14, and 17 August, respectively, 2018-2020) for both experiments. Thus, each pasture was grazed for a total of 91 days in 2018 and 2019 and 82 days in 2020. All animals were weighed on two consecutive days prior to and at the conclusion of grazing each year. Mean initial body weight (IBW) (± SE) of testers on SG was 312.5 ± 3.6 kg (n = 2; 2018), 301.2 ± 4.7 kg (n = 3; 2019) and 275.5 ± 2.0 kg (n = 3; 2020). Mean IBW (± SE) on BBIG was 292.7 ± 17.8 kg (n = 2; 2018), 269.2 ± 4.6 kg (n = 3; 2019), and 293.2 ± 8.3 kg (n = 3; 2020). Mean IBW and mean ending (E) BW for each tester was used to calculate average daily gain (ADG; the difference of EBW and IBW divided by the total number of days grazing). Grazing days of all animals, testers and grazers combined, per pasture were recorded and adjusted for area available for grazing based on closure of rested units during the course of the season to calculate animal grazing days ha^{-1} (AD; the sum of all days each animal grazed divided by number of grazeable hectares). Cattle had access to at least 60% of each pasture during the entire duration of the grazing season. Total gain ha^{-1} (GAIN) for each pasture was the product of ADG and AD each year. Animals had *ad libitum* access to mineral, water, and shade in each NWSG pasture. Animal care adhered to the UT-Institutional Animal Care and Use protocols No. 2258-0417 and No. 2258-0320.

Pasture Management and Measurements

Prior to grazing (March/April) each year, pastures were defoliated with a rotary mower and fertilized to reflect prevailing management recommendations for NWSG pastures. Each NWSG pasture was cut to a 20-cm stubble height to remove all standing, dormant biomass then fertilized with 67.3 kg N ha^{-1} yr^{-1} in the form of urea [$CO(NH_2)_2$] on 27 March 2018 and 26 May 2020 and 18.2 kg N ha^{-1} yr^{-1} on 7 May 2019. Pastures were spot-sprayed with glyphosate {N-[phosphonomethyl] glycine, isopropyl-amine salt; 41%} to remove undesirable grass species following the 2019 grazing season (September) and again in July 2020.

Botanical composition was assessed prior to and at the conclusion of grazing each year by counting desired forb plants and all NWSG tillers within four randomly placed 0.25-m^2 quadrats within each experimental unit (EU) within each NWSG pasture. Counts were taken at least 1 m from the boundary of another EU, fence, or other heavily traveled area. Forage samples were collected to determine forage nutritive values and forage mass dry matter yield and percentage throughout the growing season (only 2019 data were used) (Table 2.4). Samples were collected by clipping three randomly located 0.25-m^2 quadrats at ≥20cm for forage nutritive value and ≥5cm for forage mass within each EU. Samples were then separated for forage mass into two categories, forbs and NWSG, based on target species; non-target species (i.e., weeds) were a minor component of the sward and were not analyzed. In 2019, sampling dates during grazing coincided with the end date of each grazing treatment to reflect forage dry matter mass percentage and forage nutritive value of regrowth. Concurrent with forage sample collections, planted native forbs were categorized as not present, present and not flowering, or present and flowering within each EU. Only one plant per desired species was needed to have been observed for that species to have been considered present and not flowering or present and flowering.

Nutritive Value Analysis

Following sample separations, samples were dried at 55°C in a forced-air oven (Wisconsin Oven Corporation, East Troy, WI) for at least 72 hours and weighed for forage mass dry matter yield and percentage. Native WSG and forb species were then recombined and ground to pass a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) for forage nutritive value analysis. Nutritive value estimates of acid detergent fiber (ADF), amylase neutral detergent fiber (aNDF), crude protein (CP), and *in vitro* true dry matter digestibility following a 48-hour incubation (IVTDMD48h) were predicted via near-infrared reflectance spectroscopy (NIRS) using a SpectraStar 2600 XT-R using UScan software (Unity Scientific, Milford, MA). The 2018 Grass Hay and Mixed Hay calibrations provided by the NIRS Forage and Feed Consortium (NIRSC, Hillsboro, WI) were standardized and checked for accuracy by the Global H statistical test comparing the scanned samples against the calibration ($H < 3.0$) and are reported accordingly (Murray and Cowe, 2004).

Statistical Analysis

Response variables [NWSG tiller density, native forb plant density, forage mass dry matter yield (forbs, NWSG, and total forage), and forage nutritive value parameters (CP, aNDF, ADF, and IVTDMD48h)] were analyzed under an ANOVA model using PROC MIXED in SAS® software, Version 9.4 (SAS Institute, Cary, NC, 2013) for significant differences ($\alpha = 0.05$) among fixed effects and their interactions. For NWSG tiller and forb plant density, fixed effects were grazing treatment (Rest; early rest, middle rest, late rest, no rest, or no graze), sampling period (Period; May or September), and year (Year; 2018, 2019, or 2020) while replication was a random effect. Year was treated as a repeated factor. For forage mass dry matter yield and nutritive value parameters in 2019 fixed effects were grazing treatment per sampling period, and replication was a random effect. Mean separation was conducted using Fisher's least significant difference.

Native forb flowering data was analyzed under a Chi-Square test using PROC FREQ in SAS® software, Version 9.4 (SAS Institute, Cary, NC, 2013) for significant differences ($\alpha = 0.05$) among grazing treatments within a given sampling period pooled across years (2019 and 2020). Flowering of desired species was categorized as "yes" or "no" within a given sampling period. Only present species were incorporated into totals. Lanceleaf coreopsis was not incorporated into totals since it is characterized as a cool-season forb and therefore, would not be expected to be flowering concurrently with the other species.

RESULTS

Environmental Conditions

During the study (June 2017-September 2020), mean monthly air temperatures were similar to or above 30-year means (25 of 40 months; Figure 2.2a) as were temperatures during the growing season (April-September; 13 of 22 months). Monthly precipitation was similar to mean monthly air temperature in that 25 out of 40 months were greater than 30-year means (Figure 2.2b). However, less than half (10 of 22) of the months during the growing season had greater monthly precipitation. Following planting in June 2017, the remaining four months (including June) of the growing season were abnormally dry. While April and August 2018 followed this pattern, June, July, and September were uncharacteristically wet (97%, 22%, and 43% greater than 30-year mean, respectively). In 2019, June and July had greater than 30-year mean amounts of rainfall while September had less (92%) rainfall than average. In 2020, April and September (85% and 47%, respectively) had greater monthly precipitation than 30-year means (NOAA, 2021).

Tiller and Plant Density

Switchgrass

For SG tiller density, rest was not influential in any interaction or independently (Table 2.5). There was a two-way interaction for Year x Period ($P < 0.001$). Post-grazing 2020 (505.3 tillers m^{-2}) had the greatest mean tiller density (Figure 2.3). Switchgrass tiller density increased over time from 2017-2020 and generally increased from pre-grazing to post-grazing each year. Also, pre-grazing tiller density increased each year from the previous year as did post-grazing tiller density. Switchgrass tiller density increased from pre-grazing in 2018 to post-grazing in 2020 (148%) while forb plant density decreased. Forbs forage mass dry matter percentage averaged across no graze and no rest treatments pre-grazing consisted of 42.7% whereas post-grazing was 11.8%.

Out of the 11 native forb species analyzed for plant density, seven species were significant for at least one main effect or interaction (Table 2.5). The three-way interaction only influenced black-eyed susan (BESU; $P = 0.007$), while the two-way interactions of Year x Period and Rest x Year affected five and three species, respectively. Only PCON ($P = 0.007$) was influenced by Year while Rest and Period by themselves did not affect any species. Illinois bundleflower, Maximilian sunflower (MAXI), and upright prairie coneflower (UPRT) plant densities were not affected by any fixed effect.

Among all grazing treatments, pre-grazing 2018 had greater BESU plant density than all other Rest x Year x Period combinations (Table 2.6). Early rest and no rest (43.3 and 42.0 plants m^{-2}, respectively) had the greatest BESU plant density of all grazing treatments pre-grazing 2018; however, at this time, grazing had yet to occur on the study. Dixie ticktrefoil (DITI), lanceleaf coreopsis (LANC), and partridge pea (PPEA) plant density all had a two-way interaction for Rest x Year ($P < 0.001$, $P = 0.020$, and $P = 0.030$, respectively). The Rest x Year interaction showed DITI and PPEA plant density greatest for no graze in 2020 (16.1 plants m^{-2}) and early rest in 2019 (1.2 plants m^{-2}), respectively (Table 2.7). For both species, all other values were similar to each other. For LANC, early rest and no graze in 2019 (38.4 and 34.2 plants m^{-2}, respectively) were greater than all other combinations for 2018 and 2020. Furthermore, all grazing treatments in 2020 were lower than 2018 and 2019 for LANC plant density, which was not observed for DITI and PPEA. Dixie ticktrefoil, LANC, oxeye sunflower (OXEY), plains coreopsis (PLAC), and PPEA plant density had a two-way interaction for Year x Period (P = 0.026, P < 0.001, P = 0.003, P < 0.001, P = 0.036, respectively). All five species had the greatest plant density during pre-grazing sampling periods although not all species were greatest in the same year (Table 2.8). For DITI, PLAC, and PPEA, all other periods had similar plant densities. Plains coreopsis was greatest for pre-grazing in 2018 (32.3 plants m^{-2}), LANC and PPEA (44.8 and 0.7 plants m^{-2}, respectively) in 2019, and DITI (6.9 plants m^{-2}) in 2020. Oxeye sunflower was similar in both 2019 and 2020 (3.7 and 6.1 plants m^{-2}, respectively). Purple coneflower (PCON) plant density differed by Year (P = 0.007) with 2019 and 2020 (5.0 and 3.8 plants m^{-2}, respectively) greater than 2018 (1.9 plants m^{-2}).

Forb species were ranked based on abundance (total plants m^{-2}), persistence (plants m^{-2} remaining in 2020), flowering period, and bloom abundance ($plant^{-1}$) (Table 2.9). Lanceleaf coreopsis ranked as the best forb to plant within SG based on these results. The highest ranked annual/biennial forb was BESU. Dixie ticktrefoil ranked highest among legumes. Purple prairie clover ranked last among all forbs.

Big Bluestem/Indiangrass

For BBIG tiller density, rest was not significant independently or in any interaction (Table 2.10). There was a two-way interaction for Year x Period (P = 0.002). In 2019 and 2020, pre-grazing (581.6 and 604.5 tillers m^{-2}, respectively) had the greatest mean tiller densities with no difference between post-grazing in 2019 and 2020 (Figure 2.4). Pre- and post-grazing in 2018 had the lowest BBIG tiller density (326.1 and 341.1 tillers m^{-2}, respectively), and were similar to post-grazing in 2019 (391.1 tillers m^{-2}). Tiller density increased numerically each pre-grazing period from the previous year's post grazing counts. Each year's post-grazing densities were numerically greater than the preceding year's post-grazing density. Big bluestem/indiangrass tiller density increased from pre-grazing in 2018 to post-grazing in 2020 (43%) while forb plant density decreased. Forbs forage mass dry matter percentage averaged across no graze and no rest treatments pre-grazing consisted of 42.3% whereas post-grazing was 23.6%. Rest was not significant independently or in any interaction.

Seven of the 11 native forb species were significant for at least one main effect or interaction analyzed for plant density (Table 2.10). Dixie ticktrefoil, ILBF, and PPEA plant density was not influenced by any fixed effect. No species was significant for the three-way interaction of Rest x Year x Period. Rest x Year only influenced PCON plant density (P = 0.010). Numerically, all treatments that had been grazed decreased in plant density from 2019 to 2020 (Table 2.11). However, there was not a consistent statistical pattern observed. For the Rest x Period interaction, BESU (P = 0.047) plant density was greater at pre-grazing than post-grazing for all grazing treatments (Table 2.12). However, MAXI plant density was greatest for late rest at pre-grazing (1.4 plants m^{-2}) with all other combinations similar to one another. Year x Period (P < 0.001) affected BESU, LANC, OXEY, PCON, and PLAC plant density. For all five species, the greatest plant density was observed during pre-grazing sampling periods although not all species were greatest in the same year (Table 2.13). Plant density for pre-grazing in 2018 was greatest for BESU (10.1 plants m^{-2}) and PLAC (13.3 plants m^{-2}), whereas LANC (15.0 plants m^{-2}) and PCON (3.8 plants m^{-2}) were greatest in 2019. Oxeye sunflower plant density was greatest for pre-grazing in 2020 (5.5 plants m^{-2}). Aside from the greatest plant density for OXEY and PLAC, all other combinations were similar to one another although no plants were observed for PLAC in 2020. Upright prairie coneflower was significant for Year (P = 0.009) with 2018 and 2019 (0.9 and 1.0 plants m^{-2}, respectively) greater than 2020 (0.1 plants m^{-2}). Among the 11 forb species ranked within BBIG, DITI ranked as the best forb and legume species for abundance, persistence, and flowering. within SG based on these results. Black-eyed susan ranked highest among annual/biennial forbs. Purple prairie clover ranked last among all forbs.

Forage Mass and Nutritive Value

Switchgrass

Forage mass dry matter yield varied for forb (P = 0.016), SG (P = 0.003), and total forage (P = 0.001) between within-season rest treatments at the 20 August sampling period (Table 2.14). For all three categories, no graze had greater forage mass dry matter yield than no rest. Forage nutritive values were not affected by grazing treatments at any sampling period in 2019.

Big Bluestem/Indiangrass

Forage mass dry matter yield only differed for BBIG with respect to within-season rest treatments at every sampling period (Table 2.15). On 15 May, 1 August, and 20 August, no graze had the greatest yield among sampled within-season rest treatments (Figure 2.7). On 19 Jun and 10 July, no graze was only greater than the no rest treatments. For total forage, within-season rest treatments varied on 10 Jul, 1 August, and 20 August with no graze having greater forage mass dry matter yield than all other sampled treatments (Table 2.15; Figure 2.6).

For forage nutritive value in 2019, sampling periods were analyzed independently for the corresponding grazing treatment (Table 2.16). On 15 May, 10 July, 1 August, no nutritive value differed among within-season rest grazing treatments. Crude protein varied by grazing treatments on 19 June (P = 0.023) only. At this sampling period (the end of the early rest), no rest (99.1 g kg^{-1}) was greater than no graze (83.7 g kg^{-1}) (Figure 2.7). Early rest (93.5 g kg^{-1}) was similar to both. Amylase NDF was also significant on 19 June (P = 0.038). No rest (607.4 g kg^{-1}) had lower fiber content than no graze (655.3 g kg^{-1}) and early rest (658.1 g kg^{-1}) (Figure 2.8). No rest was also lower in ADF (356.7 g kg^{-1}) and greater in IVTDMD48h (756.6 g kg^{-1}) than no graze on August 20 (418.3, 545.9, and 637.6 g kg^{-1}, respectively) at the conclusion of the 2019 grazing season (Figures 2.9 and 2.10).

Native Forb Flowering

Switchgrass

Native forb flowering percentage was only influenced by grazing treatment on the July/August sampling period (Table 2.17). At this sampling period, late rest had 63.9% flowering of observed species while no graze and no rest had 56.3% and 21.6%, respectively (P < 0.001, x^2 = 14.80; Figure 2.11). No graze, early rest, and middle rest flowering percentage of combined species went from <5% in May to >60% in

September. Late rest flowering percentage reached 63.9% in July/August but dropped to 61.3% in September. No rest flowering percentage reached 62.5% in September.

Big Bluestem/Indiangrass

Native forb flowering percentage did not differ among grazing treatments within sampling periods (Table 2.17). Flowering percentage for all grazing treatments was below 5.7% in May and reached 88.9% by September (Figure 2.12).

Steer Performance and Pasture Productivity

Switchgrass

Due to rest periods within pastures, grazing days varied by grazed treatments and ranged from 217-334, 336-451, and 391-420 d ha^{-1} in 2018, 2019, and 2020, respectively. As expected, no rest had the greatest AD among treatments. Average daily gain of steers averaged 0.75, 0.96, and 0.97 kg d^{-1} in 2018, 2019, and 2020, respectively. Total gain averaged 204, 344, and 388 kg ha^{-1} in 2018, 2019, and 2020, respectively.

Big Bluestem/Indiangrass

In 2018-2020, animal grazing days ranged from 231-349, 336-451, and 391-420 d ha^{-1}, respectively, across all grazed treatments. Early, middle, and late rest had fewer AD than no rest. Average daily gain of steers averaged 0.87, 0.80, and 0.82 kg d^{-1} from 2018-2020, respectively. Total gain averaged 248, 288, and 325 kg ha^{-1} in 2018, 2019, and 2020, respectively.

DISCUSSION

Tiller and Plant Density

The increase in both SG and BBIG tiller density from 2018-2020 showed a shift in plant dominance toward the perennial grass species. Bonin and Tracy (2012) observed a shift in plant species dominance from native forbs to native perennial grasses from 2008-2011 in a 10-species polyculture where half of the mixture consisted of native grasses. However, all species were planted simultaneously and never grazed as opposed to interseeded into established NWSG and grazed in the current studies.

Switchgrass tiller density increased more than three times that of BBIG tiller density from 2018-2020. Big bluestem/indiangrass was more heavily grazed than SG within their respective pastures. Tomanek, Martin, and Albertson (1958) observed that big bluestem was preferentially grazed over other grasses within a mixed prairie in Kansas. Dwyer, Sims, and Pope (1964) reported that steers preferred BB over SG when grazing pure stands in Oklahoma. In 2020, more reproductive tillers were observed in the SG pasture than the BBIG pasture thus allowing for greater competition with native forbs. Lack of grazing pressure on the SG early in the season could have influenced plant maturity. The lower BBIG tiller density in 2020 showed that BBIG may not be as competitive with native forbs as SG when being grazed.

All forb species in these studies were natives, shared a common seasonality (except LANC), tended to be upright in growth habit, and did not present meaningful competition to grasses contrary to what has been reported with interseeding non-natives and/or cool-season species (Blanchet et al., 1995; Keyser et al., 2016). The decrease in forb plant density could be attributed to competition with NWSG, trampling, or being grazed by steers (Van Vuren & Bray, 1983; Hartnett et al., 1997) or wildlife. Multiple forb plants were observed to have been foraged upon throughout the course of the current studies. As previously mentioned, Bonin and Tracy (2012) found a decline in native forbs in a 10-species polyculture with native perennial grasses where half of the mixture consisted of native forbs and legumes. Four species (ILBF, PPEA, BESU, and OXEY) were the same as those used in the current studies. Sanderson et al. (2007) conducted a literature review on mixed sward pasture management and reported that close to half of the species planted within swards of greater than five species failed to persist for more than 3-4 years.

Out of the 11 forb species planted in the SG and BBIG pastures, PPCL was the only forb species that never established. Contrarily, Berg (1990) no-till drilled a five-species forb mixture into wheat [*Triticum aestivum* L.] residue and found PPCL was the most abundant forb species present in their southern Great Plains study site. Out of the 10 species that established in the SG study, UPRT, ILBF, and PPEA were the only forb species that never had ≥ 1.0 plants m^{-2}. In the BBIG pasture, MAXI, ILBF, and PPEA were the only species to have ≤ 1.0 plant m^{-2} at each pre- and post-grazing sampling period. Thus, the only one of the four legumes that was well represented was DITI.

In the SG and BBIG pastures, annual/biennial species represented the majority (73.8 and 61.3%, respectively) of the total forb plant density in 2018 (Year 2 after planting) and declined to 11.6 and 4.9% at the conclusion of grazing in 2020. Tracy and Bonin (2013) observed a similar species shift in six- and ten-species mixtures that were initially dominated by BESU (annual/biennial; USDA-NRCS 2019) to shared dominance of BESU with perennial grasses after the first two years when seeded together. They hypothesized this shift was due to the slower establishment of native grasses when compared to the short-lived biennial, BESU. Similarly, in the current studies, a decline in BESU plant density was observed but this species ranked highest among annual/biennial species for abundance. Annual (PLAC and PPEA) species were unable to consistently reseed from year-to-year and therefore, did not persist. De Cauwer and others (2005) also observed a shift in the plant community from annuals to perennials after three years when sowing seed mixtures of ≥ 63 species. Partridge pea was the only annual to increase in plant density from 2018 to 2020 in the SG study. However, this increase was negligible (0.05-0.13 plants m^{-2}) in terms of total abundance. The greatest plant density for PPEA in SG was at pre-grazing in 2019, the beginning of the third year after the initial planting (2017). This could have resulted from hard or dormant seed germination. This is not consistent with results from Ashworth et al. (2015) where PPEA was greatest in the first year of their study. Following year 1, PPEA declined in year 2 but increased from year 2 to year 3. They hypothesized this increase could have been caused by reseeding. Plains coreopsis was absent in SG and both PLAC and PPEA were absent in BBIG at the end of 2020.

Out of the seven forb species that established in both experiments, the majority were perennial forb species. Dixie ticktrefoil, LANC, and OXEY were the only perennial forbs that had a mean plant density >5 plants m^{-2} in both NWSG pastures. Lanceleaf coreopsis ranked highest for abundance in SG and BBIG while DITI ranked second in BBIG. Additionally, DITI and OXEY plant densities were greatest at pre-grazing 2020. However, this increase in forb plant density did not overwhelm SG and BBIG. This may be due to interseeding forbs into established NWSG stands as opposed to seeding both forbs and grasses simultaneously. Wagner (2020) reported that three NWSG species accounted for < 5% of the flora at the end of the first two years following a combined grass-forb planting despite NWSG consisting of 70% of the 18-species seed mix. In both NWSG pastures, DITI, OXEY, and PCON ranked highest for persistence as they had the greatest plants at the conclusion of the studies in 2020. Upright prairie coneflower was the only perennial that established but failed to persist through 2020.

Forage Mass and Nutritive Value

When there was a difference for forage mass dry matter yield for either forb, NWSG, or total forage, no graze was always greater than no rest. Also, because BBIG dry matter yield varied among within-season rest treatments at every sampling period, BBIG appeared to be more heavily grazed than SG. Stocking proportional to carrying capacity was lower for SG than for BBIG. Evidence for this is borne out from the fact that within-season rest treatments only differed for SG on 20 August, the final sampling period which followed the grazing season.

In 2019, when there was a difference for nutritive value estimates at a specific sampling period, no graze was always worse than no rest. McIntosh et al. (2016) reported greater ADF and NDF when SG was harvested at the end of the growing season for biomass vs. plots harvested previously during the growing season. This contrast in maturity is comparable to not grazing vs. grazing.

Literature on forage nutritive value of native forb:grass mixtures in the Eastern U.S. is lacking. Bonin and Tracy (2011) broadcast seeded a 10-native prairie species (five perennial grasses and five forbs, three of which were legumes) mixture into a small plot study for forage nutritive value analysis in Virginia. Four out of five of the forbs used were also utilized in the current study. When analyzing species separately, they reported CP for all ten species was similar or above 6 to 7%, noting this as the minimum for non-lactating beef cattle. However, all of their samples were harvested in August at mature growth stages. All CP values of mixed swards in the current experiments exceeded this minimum requirement.

Native Forb Flowering

Native forbs flowered throughout the duration of the grazing season. Native forb flowering percentage was not influenced by grazing treatments in BBIG. For SG, July/August was the only sampling period where flowering percentage was affected by grazing treatments with no rest having the lowest flowering percentage. With the exception of this sampling period, both SG and BBIG pastures had similar flowering percentages for all other periods. May had a low percentage of flowering warm-season forbs in both pastures (<6.8% SG and <5.7% BBIG). At this period LANC was the most prolific flowering forb. This was expected since LANC flowers from April-June (USDA-NRCS, 2002). Between June and post-grazing sampling periods, flowering percentage ranged from 40.4-88.9% for BBIG and 33.3-76.9% for SG (excluding no rest in July/August for SG). Flowering during this time of year is important for pollinator species since other sources of nectar and pollen are deficient (Wagner, 2020). Tuell et al. (2008) noted that bees could benefit from pollinator conservation projects that include native perennial species with multiple bloom periods. Besides PPCL, which never established, ILBF had the least frequent flowering. Illinois bundleflower was only observed flowering once within either study across both years. Also, this low flowering observation could have been due to the fact that ILBF had an average of only 0.08 plants m^{-2} across pre- and post-grazing sampling periods and both NWSG grasses. Berg (1990) reported that ILBF was heavily grazed by cattle in Oklahoma. Cattle may preferentially graze ILBF thus decreasing plant density and ultimately flowering percentage. Based on bloom observations, LANC and PCON had the longest flowering period in SG and while PCON had the longest in BBIG. However, MAXI ranked highest in both SG and BBIG for bloom abundance.

Steer performance

Steer ADG and pasture productivity (AD and GAIN) appeared to be comparable to those reported by other researchers. Average daily gain of steers (0.89 kg d^{-1} for SG across all treatments and all years) was comparable to those from past studies, 0.83-1.05 (Mosali et al., 2013), 0.91 (Burns & Fisher, 2013), 0.93 (Krueger & Curtis, 1979) and 0.96-1.07 (Burns et al., 1984) kg d^{-1}. In Tennessee, researchers found ADG of 0.82 and 0.96 kg d^{-1} (Backus et al., 2017) for steers grazing BBIG blend pastures at two different sites, generally similar to the 0.83 kg d^{-1} for BBIG in the current study. Animal grazing days ha^{-1} for SG and BBIG averaged 346 and 351d ha^{-1}, respectively, across all treatments and all three years. Keyser and others (2016) reported lower AD for heifers ranging from 222-330 d ha^{-1} when grazing SG with red clover and 162-240 d ha^{-1} BBIG with red clover for three years. Total gain of steers grazing SG and BBIG averaged 312 and 287 kg ha^{-1}, respectively, across all grazing treatments and three years of the studies. Backus et al. (2017) reported GAIN of steers to be 299 and 489 kg ha^{-1} for SG and 257 and 415 kg ha^{-1} for BBIG at two different sites. Other researchers reported GAIN of 146 (Krueger & Curtis, 1979) and 839 kg ha^{-1} (Burns & Fisher, 2013). Based on these results, incorporating forbs had no apparent detrimental effect on animal performance or pasture productivity.

CONCLUSIONS

Planting native forbs into a SG or BBIG pasture is a viable option for increasing plant biodiversity. During the current study, PPCL never established while ILBF was only observed flowering once despite having been seeded at the highest rate of any of the 11 forbs. Utilizing the other nine species would allow for flowering throughout the NWSG grazing season. If annuals/biennials are used in mixtures, incorporating PPEA and BESU would be preferable to using PLAC. Out of the legume species used, DITI had the greatest density and should be prioritized for diversity plantings based on these experiments. Although MAXI plant density was not as great as other species, multiple blooms plant^{-1} were observed on a consistent basis. Based on plant abundance, persistence, observed flowering periods, and bloom abundance, interseeding a 6-species polyculture of BESU, DITI, LANC, MAXI, OXEY, and PCON could allow for plant biodiversity while providing ample blooms during the NWSG grazing season.

Native WSG and forbs persisted after three years of grazing. Increasing the number of grazers early in the grazing season, particularly for SG, would aid in keeping the NWSG at a manageable height that may aid in forb persistence. However, adding grazers would also increase hoof traffic that could increase forb mortality. Since grazing treatment was not influential for forb plant density or NWSG tiller density, persistence of these species does not appear to require rotational grazing.

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APPENDIX II

Table 2.1. Annual soil tests (0-15 cm; Mehlich 1) for switchgrass and big bluestem/indiangrass pastures planted with an 11-species forb blend at the University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN, in 2018-2020.

	Switchgrass			Big bluestem/Indiangrass		
Year	P	K	pH	P	K	pH
	----------------------------------kg ha^{-1}----------------------------------					
2018	12.7	58.1	6.4	14.0	78.7	6.4
2019	12.3	111.0	6.4	12.3	85.1	6.6
2020	16.8	68.4	6.4	12.3	70.6	6.5

Table 2.2. Native warm-season forbs species planted in a mixture for native grass pasture grazing experiments, June 2017, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

Common Name†	Latin Name	Total kg ha^{-1}	A/B/P‡
Partridge pea§	*Chamaecrista fasciculata*	0.56	A
Lanceleaf coreopsis	*Coreopsis lanceolata*	1.12	P
Plains Coreopsis	*Coreopsis tinctoria*	0.56	A
Purple Prairie Clover	*Dalea purpurea*	0.56	P
Illinois Bundleflower, Midwestern U.S. Eco§	*Desmanthus illinoensis*	1.26	P
Dixie Ticktrefoil, AL Eco§	*Desmodium tortuosum*	0.56	P
Purple Coneflower	*Echinacea purpurea*	0.70	P
Maximilian Sunflower	*Helianthus maximilianii*	0.56	P
Oxeye Sunflower (False Sunflower)	*Heliopsis helianthoides*	0.28	P
Upright Prairie Coneflower	*Ratibida columnifera*	0.28	P
Black-Eyed Susan, AL Eco	*Rudbeckia hirta*	0.56	A/B/P
	Total	7.00	

†Seed purchased from Ernst Conservation Seeds, Inc., Meadville, PA
‡A/B/P = annual, biennial, or perennial species
§Legumes

Table 2.3. Grazing treatment dates within switchgrass and big bluestem/indiangrass pastures for native grass pasture grazing experiments, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

	Year		
Treatment	2018	2019	2020
Early Rest	31 May – 20 Jun	30 May – 19 Jun	9 Jun – 26 Jun
Middle Rest	21 Jun – 11 Jul	20 Jun – 10 Jul	27 Jun – 17 Jul
Late Rest	12 Jul – 2 Aug	11 Jul – 31 Jul	18 Jul – 7 Aug
No Rest	17 May – 15 Aug	16 May – 14 Aug	28 May – 17 Aug
No Graze	17 May – 15 Aug	16 May – 14 Aug	28 May – 17 Aug

Table 2.4. Sampling period dates for grazing treatments within switchgrass and big bluestem/indiangrass pastures for native grass pasture grazing experiments planted with an 11-species forb blend, 2019-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

	Switchgrass		Big bluestem/Indiangrass	
Treatment	2019†	2020†	2019	2020
Early Rest	19 Jun	25/26 Jun	19 Jun	24/25 Jun
Middle Rest	9 Jul	15/16 Jul	10 Jul	14/15 Jul
Late Rest	31 Jul	7 Aug	1 Aug	5 Aug
No Rest	14 May 19 Jun 9 Jul 31 Jul 19 Aug	27/28 May 25/26 Jun 15/16 Jul 7 Aug 20 Aug	14 May 19 Jun 10 Jul 1 Aug 20 Aug	27/28 May 24/25 Jun 14/15 Jul 5 Aug 19 Aug
No Graze	14 May 19 Jun 9 Jul 31 Jul 19 Aug	27/28 May 25/26 Jun 15/16 Jul 7 Aug 20 Aug	14 May 21 Jun 10 Jul 1 Aug 20 Aug	27/28 May 24/25 Jun 14/15 Jul 5 Aug 19 Aug

†In 2019 and 2020, sampling dates coordinated with the ending of each rest period for switchgrass and big bluestem/indiangrass pastures. No Rest and No Graze treatments were sampled each time as control comparisons. Sampling height for forage nutritive values was ≥20 cm and ≥5 cm for forage mass dry matter percentage. Only 2019 data were used and available at time of document publication.

Table 2.5. Mixed-effects ANOVA model for switchgrass tiller density and plant density of 11 native forb species within native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

	SG†		BESU		DITI		ILBF		LANC		MAXI	
Effect	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
Rest‡	2.54	0.059	2.63	0.054	4.02	**0.012**	0.79	0.540	0.46	0.763	0.04	0.997
Year	72.77	**<0.001**	56.50	**<0.001**	5.39	0.092	2.06	0.136	104.01	**<0.001**	1.91	0.233
Rest x Year	1.54	0.165	2.96	**0.009**	4.21	**<0.001**	0.95	0.480	2.50	**0.020**	0.20	0.990
Period	28.33	**<0.001**	78.69	**<0.001**	1.42	0.245	1.44	0.236	30.16	**<0.001**	3.73	0.065
Rest x Period	0.53	0.714	1.17	0.345	0.51	0.727	1.61	0.186	0.51	0.729	0.39	0.812
Year x Period	11.39	**<0.001**	81.83	**<0.001**	4.01	**0.026**	0.87	0.426	28.81	**<0.001**	2.39	0.105
Rest x Year x Period	1.06	0.406	3.05	**0.007**	0.96	0.481	0.57	0.799	0.75	0.646	1.02	0.438

	OXEY		PCON		PLAC		PPCL		PPEA		UPRT	
Effect	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
Rest‡	0.38	0.823	1.59	0.201	1.76	0.162	-	-	2.48	0.059	1.94	0.123
Year	5.92	**0.043**	14.36	**0.007**	106.46	**<0.001**	-	-	3.79	0.069	1.77	0.180
Rest x Year	0.48	0.868	1.14	0.351	1.34	0.248	-	**-**	2.34	**0.030**	0.57	0.798
Period	8.02	**0.008**	0.06	0.816	232.82	**<0.001**	-	-	0.96	0.333	0.00	0.966
Rest x Period	0.24	0.915	1.39	0.260	1.69	0.179	-	-	0.57	0.683	0.19	0.941
Year x Period	6.76	**0.003**	2.71	0.076	142.46	**<0.001**	-	**-**	3.52	**0.036**	0.05	0.948
Rest x Year x Period	0.58	0.791	1.72	0.114	1.35	0.243	-	-	0.66	0.728	0.31	0.961

†SG = switchgrass, BESU = black-eyed susan, DITI = dixie ticktrefoil, ILBF = Illinois bundleflower, LANC = lanceleaf coreopsis, MAXI = maximilian sunflower, OXEY = oxeye sunflower, PCON = purple coneflower, PLAC = plains coreopsis, PPCL = purple prairie clover, PPEA = partridge pea, UPRT = upright coneflower

‡Rest = Grazing treatment (no rest, early rest, middle rest, late rest, or no graze); Year = 2018, 2019, or 2020; Period = pre-(May) or post-grazing (late August/September)

Table 2.6. Black-eyed susan mean plant density (plants m^{-2}) in a switchgrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

	2018		2019		2020	
Effect†	Pre	Post	Pre	Post	Pre	Post
	----------plants m^{-2}----------					
No Rest	42.0 a*	2.8 d	2.0 d	1.0 d	0.3 d	1.3 d
Early Rest	43.3 a	1.8 d	3.5 d	7.3 d	1.1 d	1.9 d
Middle Rest	30.5 b	1.8 d	1.0 d	3.0 d	7.3 d	0.8 d
Late Rest	19.0 c	2.5 d	0.3 d	0.5 d	0.9 d	0.0 d
No Graze	22.8 bc	0.3 d	6.5 d	4.2 d	6.6 d	1.5 d

*Different letters indicate significant difference at $\alpha = 0.05$ for Rest x Year x Period interaction (Fisher's least significant difference).

†Grazing treatment (no rest, early rest, middle rest, late rest, or no graze); Year = 2018, 2019, or 2020; Period = Pre = pre-grazing (May) or Post = post-grazing (late August/September)

Table 2.7. Mean plant density (plants m^{-2}) for DITI, LANC, and PPEA for grazing treatment by year in a switchgrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

	DITI†			LANC			PPEA		
Effect‡	2018	2019	2020	2018	2019	2020	2018	2019	2020
	------------------------------plants m^{-2}------------------------------								
No Rest	1.3 b*	1.3 b	0.5 b	15.5 e	31.3 ab	1.0 f	0.0 b	0.1 b	0.0 b
Early Rest	0.8 b	0.7 b	0.6 b	19.3 de	38.4 a	0.3 f	0.1 b	1.2 a	0.0 b
Middle Rest	1.4 b	0.5 b	1.3 b	24.6 bcd	23.0 b-e§	3.0 f	0.1 b	0.0 b	0.0 b
Late Rest	0.4 b	1.8 b	4.5 b	22.1 cde	29.6 abc	2.8 f	0.3 b	0.4 b	0.2 b
No Graze	0.0 b	3.7 b	16.1 a	14.3 e	34.2 a	1.9 f	0.1 b	0.4 b	0.5 b

†DITI = dixie ticktrefoil, LANC = lanceleaf coreopsis, PPEA = partridge pea

‡Grazing treatment (no rest, early rest, middle rest, late rest, or no graze); Year = 2018, 2019, or 2020

§Letter groups consisting of four or more sequential letters are written with the first and last letter with a dash in between

*Different letters within a species indicate significant difference at $\alpha = 0.05$ for Rest x Year interaction (Fisher's least significant difference).

Table 2.8. Mean plant density (plants m^{-2}) for DITI, LANC, OXEY, PLAC, and PPEA for period by year in a switchgrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

Period/Year	DITI†	LANC	OXEY	PLAC	PPEA
	----------------------------plants m^{-2}	-------------------------			
Pre-grazing 2018‡	0.8 b*	19.6 b	0.4 c	32.3 a	0.1 b
Post-grazing 2018	0.8 b	18.8 b	1.0 c	0.4 b	0.2 b
Pre-grazing 2019	1.2 b	44.8 a	3.7 ab	1.1 b	0.7 a
Post-grazing 2019	2.0 b	17.8 b	3.0 bc	0.0 b	0.2 b
Pre-grazing 2020	6.9 a	2.4 c	6.1 a	1.7 b	0.1 b
Post-grazing 2020	2.2 b	1.2 c	2.1 bc	0.0 b	0.1 b

†DITI = dixie ticktrefoil, LANC = lanceleaf coreopsis, OXEY = oxeye sunflower, PLAC = plains coreopsis, PPEA = partridge pea

‡Pre-grazing 2018, 2019, 2020 = May; Post-grazing 2018, 2019 = September; Post-grazing 2020 = August

*Different letters within a column indicate significant difference at $\alpha = 0.05$ for Year x Period interaction (Fisher's least significant difference).

Table 2.9. Forb species overall rank for abundance, persistence, and flowering for 11 native forb species planted within switchgrass and big bluestem/indiangrass pastures for native grass pasture grazing experiments, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

Switchgrass								
Forb Species†	Abundance (total plants m^{-2})‡	2020 Plants m^{-2}§	Abundance Rank	2020 Plants Rank	Flowering Period Rank	Blooms $plant^{-1}$ Rank	Rank Average	Overall Rank⁋
BESU	43.4	1.1	2	5	2	5	3.5	**4**
DITI	13.8	2.2	6	2	6	2	4.0	**5**
ILBF	0.3	0.1	10	7	7	6	7.5	**10**
LANC	104.5	1.2	1	4	1	3	2.3	**1**
MAXI	5.0	0.7	7	6	5	1	4.8	**6**
OXEY	16.2	2.1	5	3	2	4	3.5	**3**
PCON	21.4	3.1	4	1	1	5	2.8	**2**
PLAC	35.4	0.0	3	8	4	5	5.0	**7**
PPCL	0.0	0.0	11	8	7	7	8.3	**11**
PPEA	1.4	0.1	8	7	3	4	5.5	**8**
UPRT	1.2	0.0	9	8	2	5	6.0	**9**
Big bluestem/Indiangrass								
Forb Species†	Abundance (total plants m^{-2})‡	2020 Plants m^{-2}§	Abundance Rank	2020 Plants Rank	Flowering Period Rank	Blooms $plant^{-1}$ Rank	Rank Average	Overall Rank⁋
BESU	17.9	0.5	3	4	2	5	3.5	**4**
DITI	34.1	5.8	2	1	5	2	2.5	**1**
ILBF	0.6	0.1	9	6	6	6	6.8	**10**
LANC	42.3	0.4	1	5	2	3	2.8	**2**
MAXI	1.9	0.1	8	6	4	1	4.8	**6**
OXEY	11.5	1.2	6	3	2	4	3.8	**5**
PCON	11.6	1.3	5	2	1	5	3.3	**3**
PLAC	16.0	0.0	4	7	3	5	4.8	**7**
PPCL	0.0	0.0	11	7	7	7	8.0	**11**
PPEA	0.5	0.0	10	7	3	4	6.0	**9**
UPRT	3.9	0.0	7	7	2	5	5.3	**8**

†BESU = black-eyed susan, DITI = dixie ticktrefoil, ILBF = Illinois bundleflower, LANC = lanceleaf coreopsis, MAXI = maximilian sunflower, OXEY = oxeye sunflower, PCON = purple coneflower, PLAC = plains coreopsis, PPCL = purple prairie clover, PPEA = partridge pea, UPRT = upright coneflower

‡Sum of all plants m^{-2} counted at six sampling periods (May and August/September 2018, 2019, and 2020)

§September 2020 Plants m^{-2}

⁋Overall Rank (1-11; 1=best, 11=worst) based on Rank Average; if two Rank Averages were the same, then the species with the greatest September 2020 plant m^{-2} ranked better

Table 2.10. Mixed-effects ANOVA model for big bluestem/indiangrass tiller density and plant density of 11 native forb species for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

	BBIG†		BESU		DITI		ILBF		LANC		MAXI	
Effect	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
Rest‡	0.48	0.747	2.69	0.050§	0.50	0.735	0.38	0.822	0.80	0.534	1.51	0.217
Year	13.88	**0.002**	14.97	**0.006**	3.85	0.080	0.22	0.805	22.72	**<0.001**	1.58	0.214
Rest x Year	1.14	0.354	1.50	0.187	1.09	0.383	1.66	0.129	0.74	0.658	1.10	0.375
Period	9.51	**0.004**	80.89	**<0.001**	0.82	0.372	0.00	0.992	6.21	**0.017**	1.53	0.223
Rest x Period	0.43	0.786	2.74	**0.047**	0.34	0.852	0.72	0.582	0.20	0.937	3.91	**0.009**
Year x Period	7.26	**0.002**	17.49	**<0.001**	0.88	0.419	0.62	0.541	9.07	**<0.001**	1.64	0.202
Rest x Year x Period	1.25	0.288	1.30	0.267	0.80	0.606	1.49	0.183	1.76	0.102	1.28	0.271

	OXEY		PCON		PLAC		PPCL		PPEA		UPRT	
Effect	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
Rest‡	1.04	0.409	3.34	**0.018**	0.22	0.927	-	-	0.49	0.744	2.40	0.065
Year	3.68	0.076	2.81	0.111	16.96	**<0.001**	-	-	1.42	0.290	8.51	**0.009**
Rest x Year	1.34	0.247	2.81	**0.010**	0.34	0.945	-	-	0.73	0.661	0.71	0.683
Period	4.37	**0.047**	4.78	**0.034**	39.33	**<0.001**	-	-	0.20	0.659	2.03	0.161
Rest x Period	1.72	0.177	0.61	0.658	0.47	0.758	-	-	1.66	0.173	0.69	0.603
Year x Period	10.12	**<0.001**	11.10	**<0.001**	21.39	**<0.001**	-	-	0.05	0.954	0.62	0.541
Rest x Year x Period	0.85	0.565	1.39	0.222	0.47	0.868	-	-	1.30	0.259	0.70	0.690

†BBIG = big bluestem/indiangrass, BESU = black-eyed susan, DITI = dixie ticktrefoil, ILBF = Illinois bundleflower, LANC = lanceleaf coreopsis, MAXI = maximilian sunflower, OXEY = oxeye sunflower, PCON = purple coneflower, PLAC = plains coreopsis, PPCL = purple prairie clover, PPEA = partridge pea, UPRT = upright coneflower

‡Rest = Grazing treatment (no rest, early rest, middle rest, late rest, or no graze); Year = 2018, 2019, or 2020; Period = pre- (May) or post-grazing (late August/September)

§Rest for BESU is *P* = 0.0501

Table 2.11. Mean plant density (plants m^{-2}) for PCON for grazing treatment by year in a big bluestem/indiangrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

Effect†	2018	2019	2020
	---------------plants m^{-2}-------------		
No Rest	3.3 ab*	4.0 a	0.6 f
Early Rest	1.4 c-f‡	1.0 ef	0.8 f
Middle Rest	0.9 ef	2.8 a-d	2.5 a-e
Late Rest	1.5 c-f	2.9 abc	1.1 def
No Graze	1.9 b-f	1.9 b-f	2.5 a-e

†Grazing treatment (no rest, early rest, middle rest, late rest, or no graze); Year = 2018, 2019, or 2020

‡Letter groups consisting of four or more sequential letters are written with the first and last letter with a dash in between

*Different letters indicate significant difference at α = 0.05 for Rest x Year interaction (Fisher's least significant difference).

Table 2.12. Mean plant density (plants m^{-2}) for BESU and MAXI for grazing treatment by period in a big bluestem/indiangrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

	BESU		MAXI	
Effect†	Pre	Post	Pre	Post
	----------------------plants m^{-2}----------------------			
No Rest	3.5 c*	0.5 d	0.6 b	0.1 b
Early Rest	8.1 a	0.8 d	0.0 b	0.4 b
Middle Rest	3.7 c	0.9 d	0.0 b	0.4 b
Late Rest	5.2 bc	0.2 d	1.4 a	0.0 b
No Graze	6.7 ab	0.4 d	0.1 b	0.1 b

†Grazing treatment (no rest, early rest, middle rest, late rest, or no graze); Period = Pre = pre-grazing (May) or Post = post-grazing (late August/September)

*Different letters within a species indicate significant difference at α = 0.05 for Rest x Period interaction (Fisher's least significant difference).

Table 2.13. Mean plant density (plants m^{-2}) for BESU, LANC, OXEY, PCON, and PLAC for period by year in a big bluestem/indiangrass pasture for native grass pasture grazing experiment, 2018-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

Period/Year	BESU†	LANC	OXEY	PCON	PLAC
	----------------------------plants m^{-2}	-------------------------			
Pre-grazing 2018‡	10.1 a*	9.6 bc	0.4 b	1.3 c	13.3 a
Post-grazing 2018	0.7 c	10.4 b	1.7 b	2.3 b	0.5 b
Pre-grazing 2019	4.3 b	15.0 a	1.7 b	3.8 a	2.0 b
Post-grazing 2019	0.5 c	6.0 c	1.0 b	1.3 bc	0.3 b
Pre-grazing 2020	1.8 c	0.9 d	5.5 a	1.7 bc	0.0 b
Post-grazing 2020	0.5 c	0.4 d	1.2 b	1.3 bc	0.0 b

†BESU = black-eyed susan, LANC = lanceleaf coreopsis, OXEY = oxeye sunflower, PCON = purple coneflower, PLAC = plains coreopsis

‡Pre-grazing 2018, 2019, 2020 = May; Post-grazing 2018, 2019 = September; Post-grazing 2020 = August/September

*Different letters within a column indicate significant difference at $\alpha = 0.05$ for Year x Period interaction (Fisher's least significant difference).

Table 2.14. Mixed-effects ANOVA model for forb, switchgrass, and total forage mass across within-season rest treatments sampled within sampling date in a switchgrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

	Sampling Date†									
	15-May		19-Jun		10-Jul		1-Aug		20-Aug	
Forage Mass Category	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
Forb	0.14	0.966	0.42	0.672	0.00	0.998	2.23	0.170	12.66	**0.016**
Switchgrass	1.56	0.244	2.59	0.154	0.15	0.859	4.45	0.050‡	27.64	**0.003**
Total	0.63	0.649	2.48	0.164	0.04	0.964	5.43	**0.032**	45.26	**0.001**

†On May 15, early rest, middle rest, late rest, no rest, and no graze were sampled prior to grazing. On June 19, early rest, no rest, and no graze were compared. On July 10, middle rest, no rest, and no graze were analyzed for differences. On August 1, late rest, no rest, and no graze were sampled. On August 20, only no rest and no graze were compared at the conclusion of the grazing season.
‡$P = 0.0502$

Table 2.15. Mixed-effects ANOVA model for forb, big bluestem/indiangrass, and total forage mass across within-season rest treatments sampled within sampling date in a big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

	Sampling Date†									
	15-May		19-Jun		10-Jul		1-Aug		20-Aug	
Forage Mass Category	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
Forb	0.62	0.656	0.36	0.710	2.58	0.130	0.07	0.937	0.03	0.875
Big bluestem/Indiangrass	6.45	**0.004**	6.62	**0.017**	5.58	**0.027**	6.56	**0.018**	9.65	**0.021**
Total	1.81	0.183	3.90	0.060	13.64	**0.002**	7.18	**0.014**	21.50	**0.004**

†On May 15, early rest, middle rest, late rest, no rest, and no graze were sampled prior to grazing. On June 19, early rest, no rest, and no graze were compared. On July 10, middle rest, no rest, and no graze were analyzed for differences. On August 1, late rest, no rest, and no graze were sampled. On August 20, only no rest and no graze were compared at the conclusion of the grazing season.

Table 2.16. Mixed-effects ANOVA model for forage nutritive value parameters across within-rest treatments sampled within sampling date in a big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

Nutritive Value Parameter	Sampling Date†									
	15-May		19-Jun		10-Jul		1-Aug		20-Aug	
	F value	P > F	F value	P > F	F value	P > F	F value	P > F	F value	P > F
CP‡	0.56	0.697	5.93	**0.023**	0.40	0.683	1.96	0.197	4.77	0.094
ADF	0.53	0.718	1.70	0.237	1.90	0.219	2.98	0.102	10.07	**0.034**
aNDF	1.97	0.151	4.79	**0.038**	0.78	0.493	0.83	0.468	1.76	0.255
IVTDMD48H	1.28	0.321	2.80	0.114	0.77	0.501	2.66	0.124	9.43	**0.037**

†On May 15, early rest, middle rest, late rest, no rest, and no graze were sampled prior to grazing. On June 19, early rest, no rest, and no graze were compared. On July 10, middle rest, no rest, and no graze were analyzed for differences. On August 1, late rest, no rest, and no graze were sampled. On August 20, only no rest and no graze were compared at the conclusion of the grazing season.

‡CP = crude protein; ADF = acid detergent fiber; aNDF = amylase neutral detergent fiber; IVDMD48H = *in vitro* true dry matter digestibility following 48-hour incubation

Table 2.17. Chi-square test for native forb flowering of an 11-species forb blend planted within switchgrass and big bluestem/indiangrass pastures for native grass pasture grazing experiments, 2019-2020, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN.

	Switchgrass			
Sampling Period†	x^2	P-Value	n	df
Pre-grazing	2.31	0.678	232	4
Early Rest	3.35	0.187	155	2
Middle Rest	0.76	0.685	133	2
Late Rest	14.80	**<0.001**	105	2
Post-grazing	3.15	0.533	193	4

	Big bluestem/Indiangrass			
Sampling Period	x^2	P-Value	n	df
Pre-grazing	1.59	0.810	247	4
Early Rest	1.90	0.387	166	2
Middle Rest	0.09	0.955	164	2
Late Rest	1.58	0.454	152	2
Post-grazing	5.82	0.213	234	4

†Sampling period = Pre-grazing (May), Early Rest (June), Middle Rest (July), Late Rest (July/August), Post-grazing (August/ September)

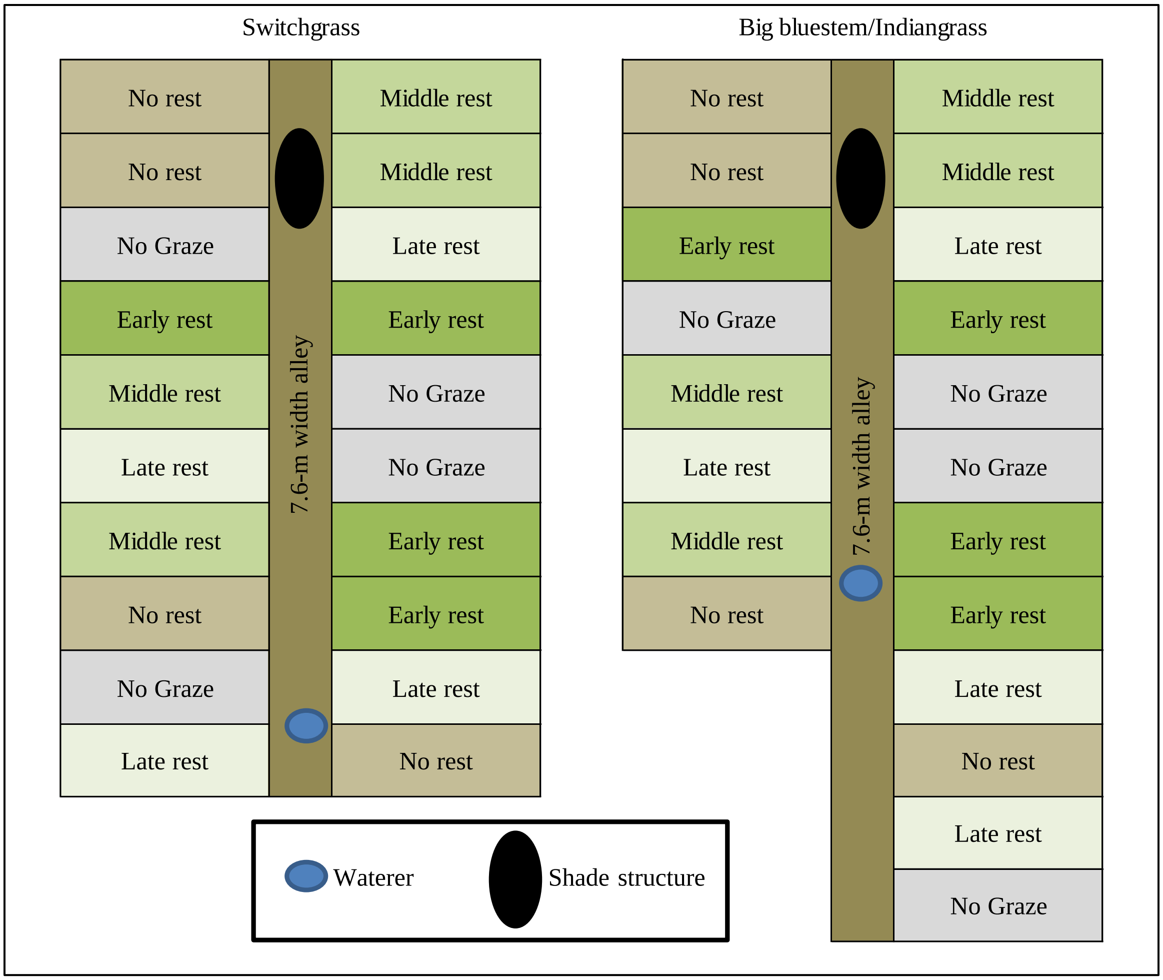

Figure 2.1 Field layout of five grazing treatments based on the timing of within-season rest (no rest, early rest, middle rest, late rest, and no grazing control) arranged in a completely randomized design with four replicates (n = 20 plots) within each of the two, 1.2-ha pastures (switchgrass and big bluestem/indiangrass mixture) interseeded with an 11-species biodiversity mixture of native forbs at the University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN, 2017-2020.

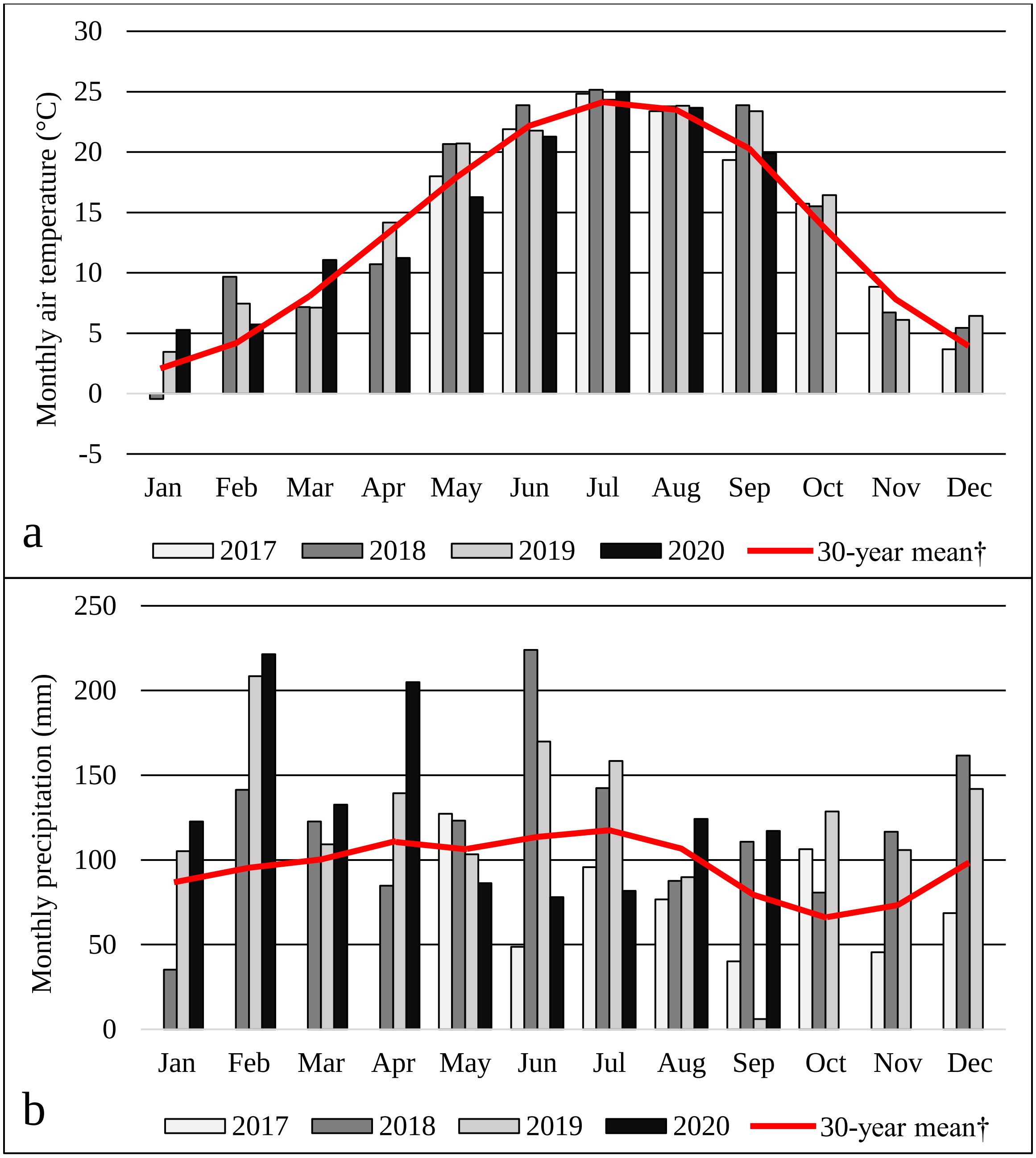

Figure. 2.2. (a) Mean monthly air temperature (°C) and 30-year mean and (b) total monthly precipitation (mm) and 30-year mean for University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN, 2017-2020. †Some months' data are missing in overall 30-year mean from 1991-2020.

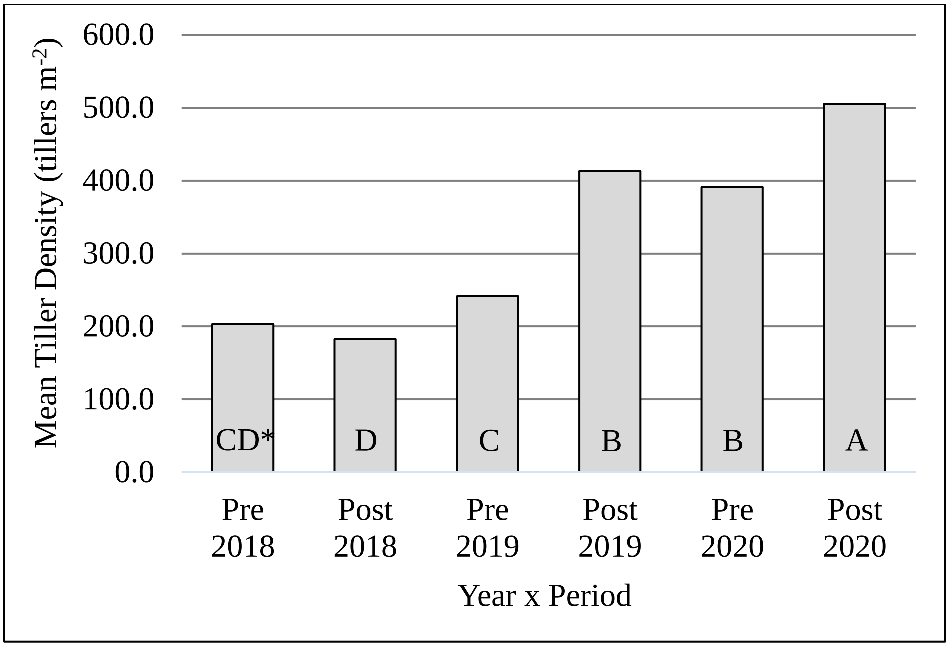

Figure 2.3. Mean tiller density (tillers m^{-2}) for switchgrass by period within year (2018-2020) at the University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. *Different letters indicate significant difference at $\alpha = 0.05$ for Year x Period interaction (Fisher's least significant difference).

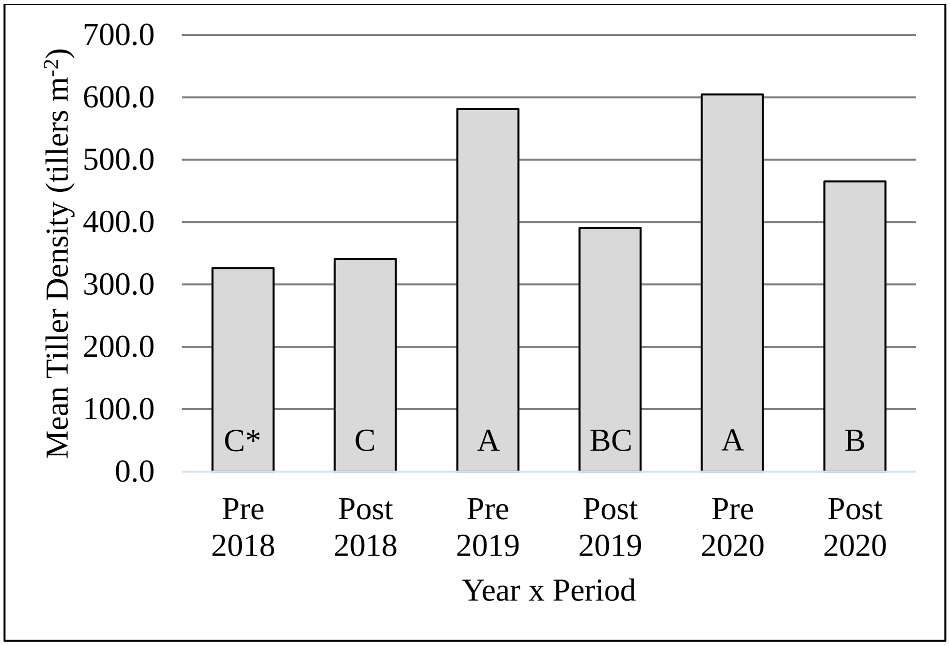

Figure 2.4. Mean tiller density (tillers m^{-2}) for big bluestem/indiangrass by period within year (2018-2020) at the University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. *Different letters indicate significant difference at $\alpha = 0.05$ for Year x Period interaction (Fisher's least significant difference).

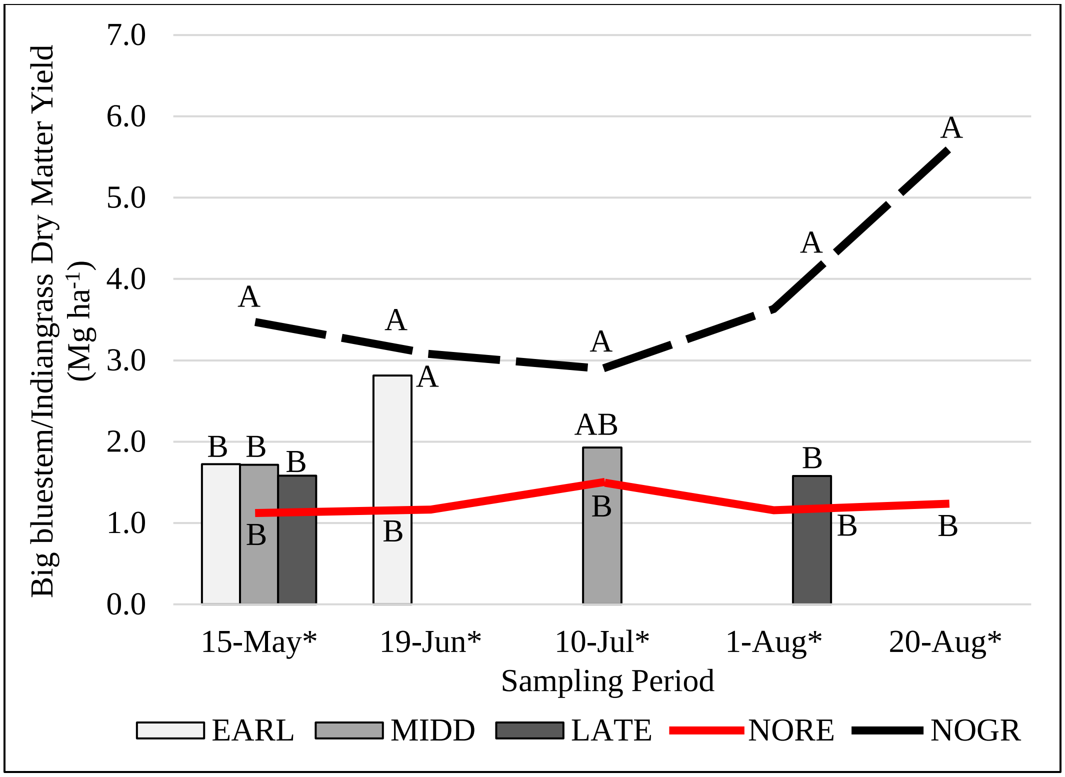

Figure 2.5. Mean big bluetem/indiangrass dry matter yield (Mg ha^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, $\alpha = 0.05$

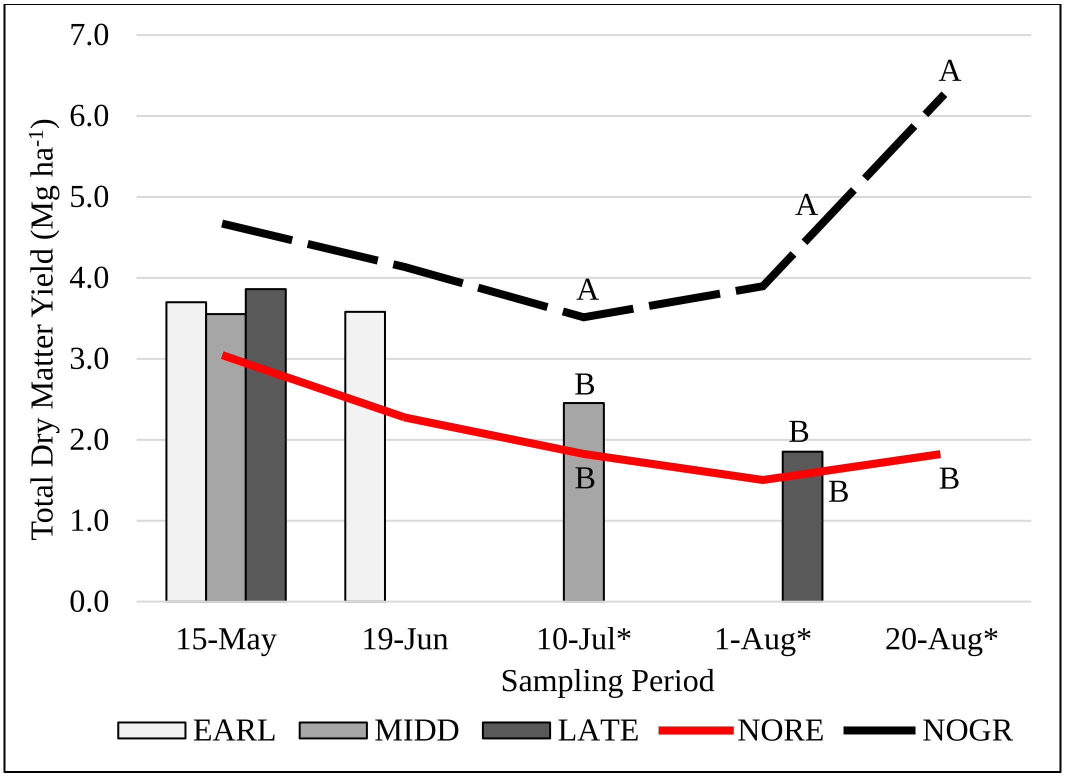

Figure 2.6. Mean total forage dry matter yield (Mg ha^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, $\alpha = 0.05$

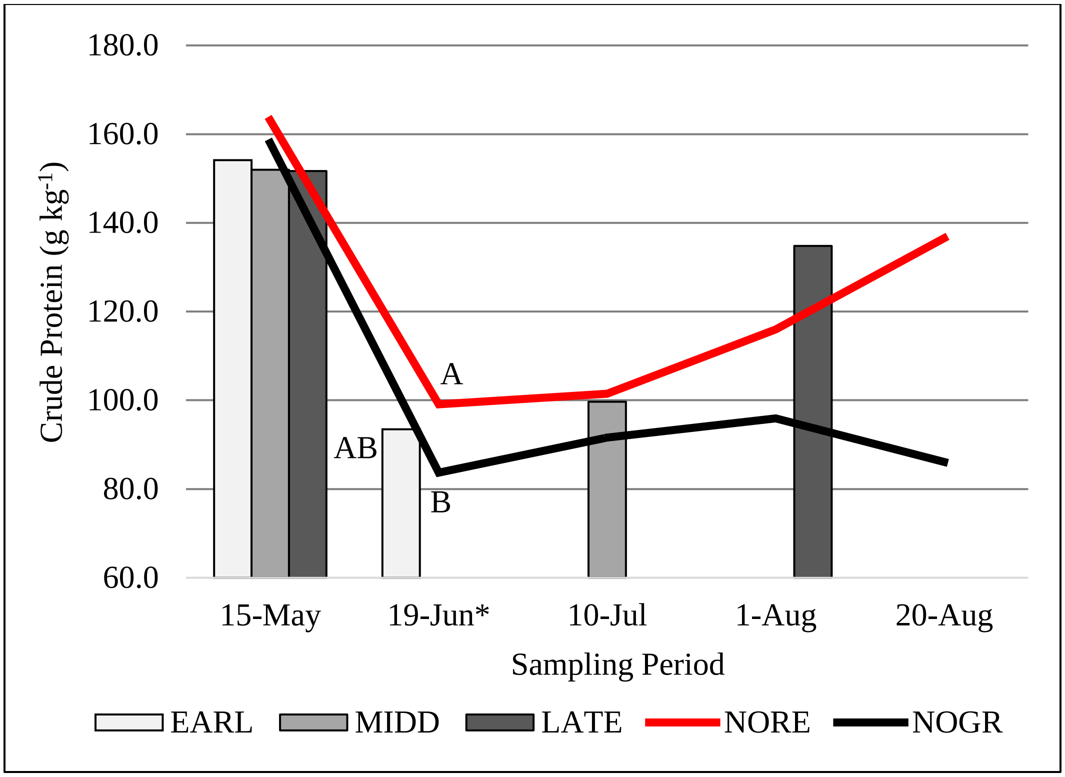

Figure 2.7. Mean crude protein (g kg^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, α = 0.05

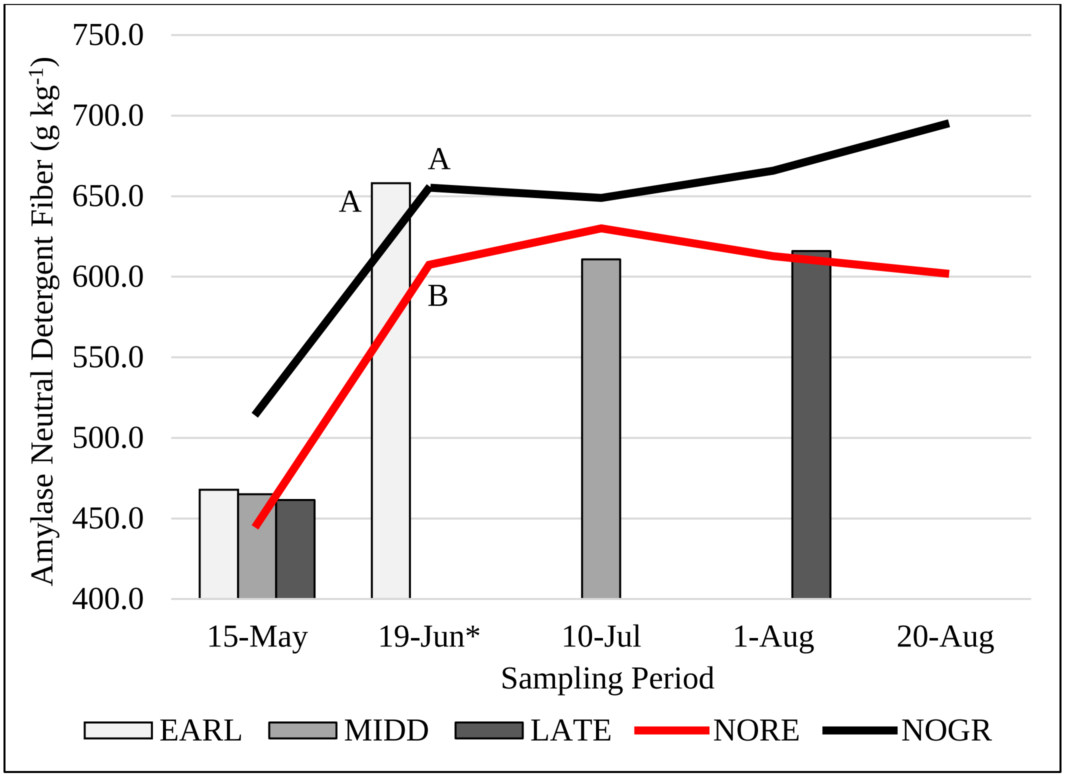

Figure 2.8. Mean amylase neutral detergent fiber (g kg^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, $\alpha = 0.05$

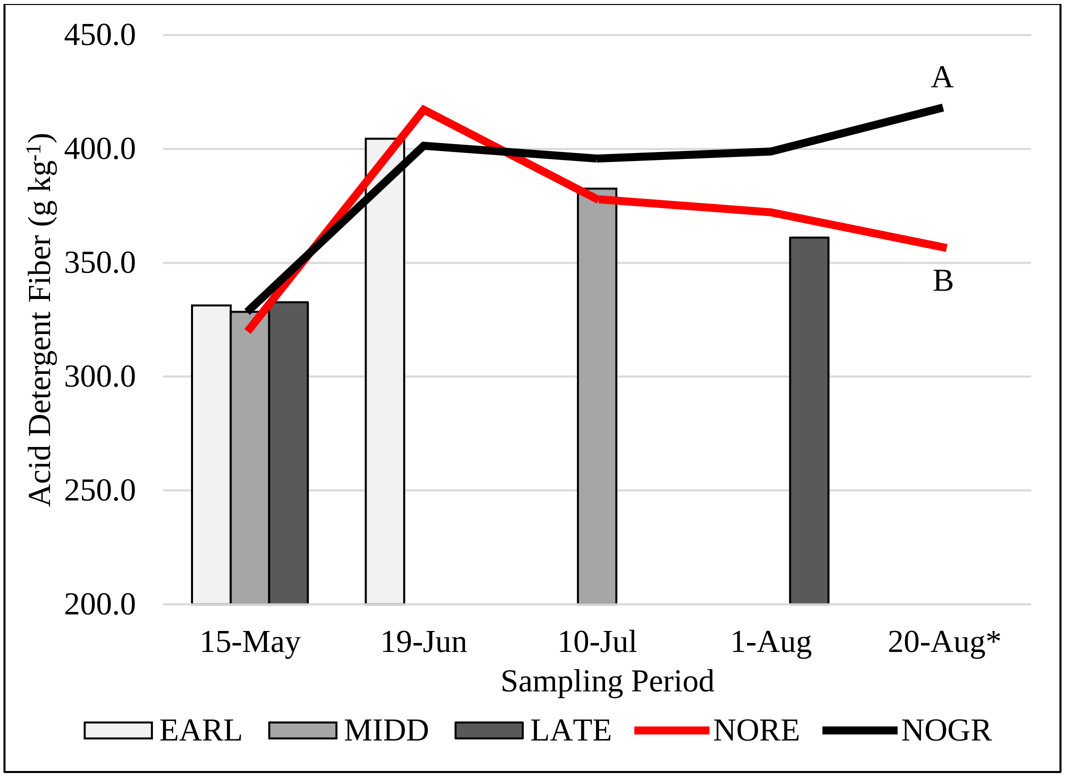

Figure 2.9. Mean acid detergent fiber (g kg^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, $\alpha = 0.05$

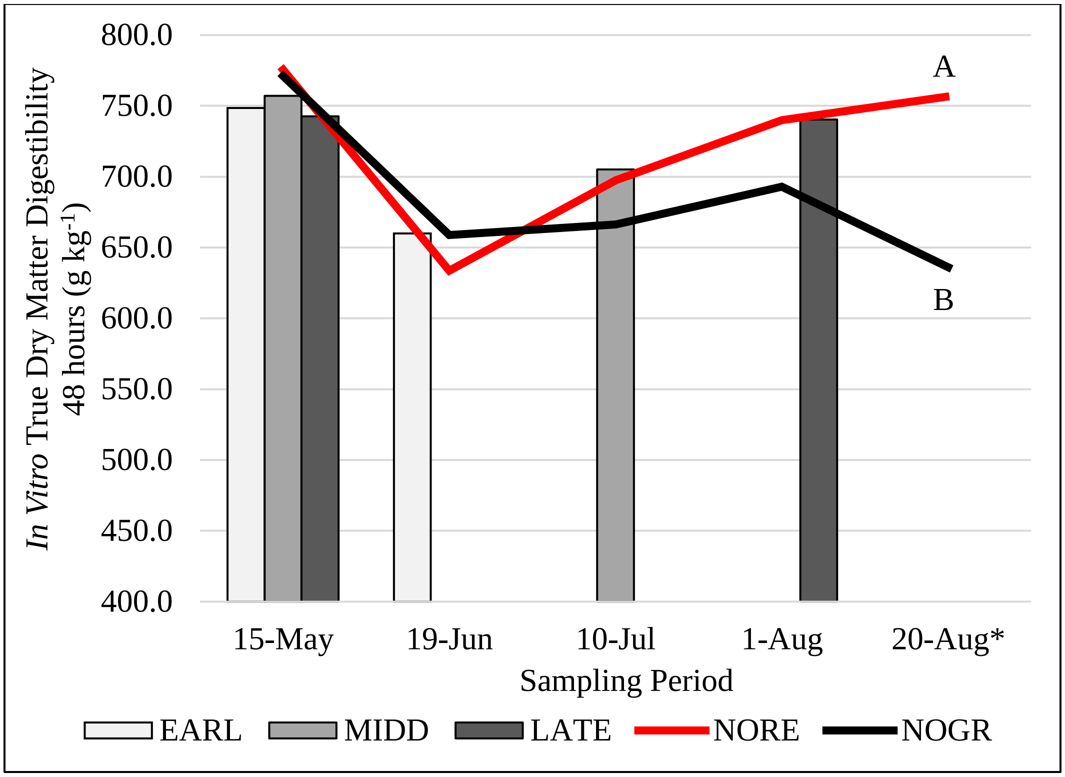

Figure 2.10. Mean *in vitro* true dry matter digestibility 48 hours (g kg^{-1}) of big bluestem/indiangrass and native forb pasture for native grass pasture grazing experiment, 2019, University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. Grazing treatments were early rest (EARL), middle rest (MIDD), late rest (LATE), no rest (NORE), and no graze (NOGR). *Significant difference among grazing treatments, α = 0.05

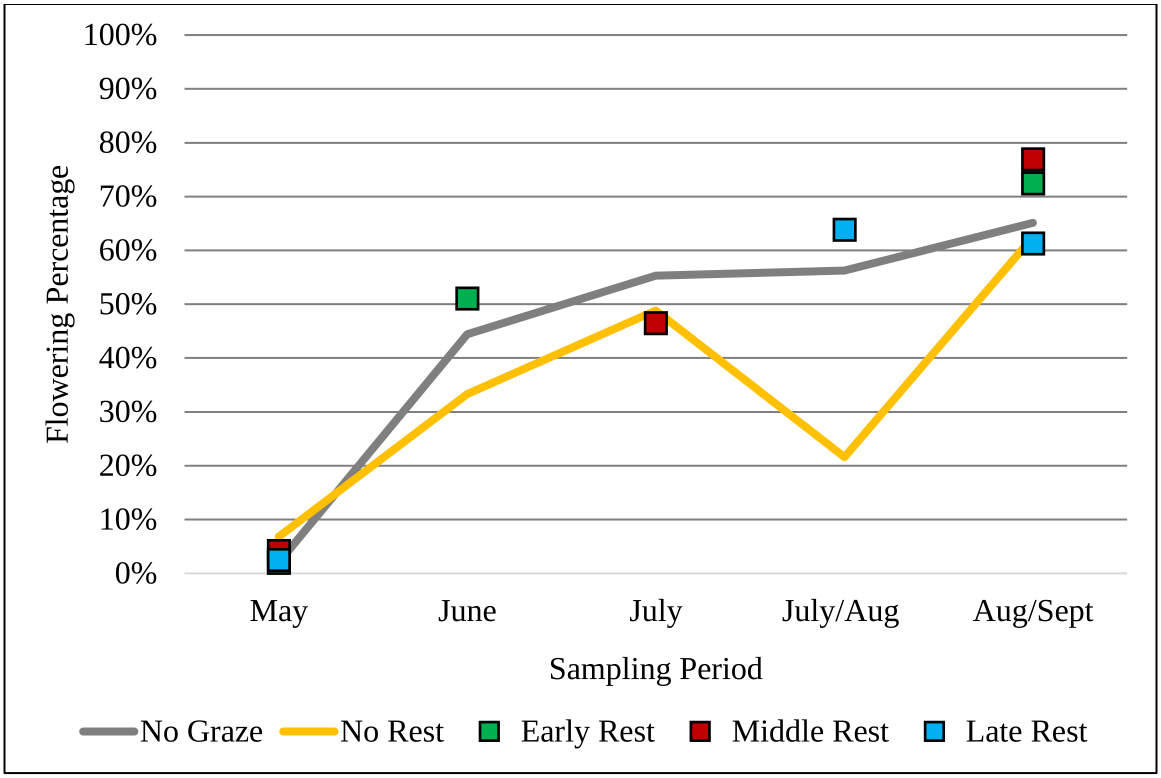

Figure 2.11. Flowering percentage of 10 native warm-season forb species pooled across years (2019-2020) at the same sampling periods in a switchgrass pasture for native grass pasture grazing experiment at the University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. †Flowering percentage compared across grazing treatments within sampling periods. All sampling periods compared no graze and no rest to the respective sampled grazing treatment. May = early rest, middle rest, and late rest; June = early rest; July = middle rest; July/Aug = late rest; Aug/Sept = early rest, middle rest, and late rest.

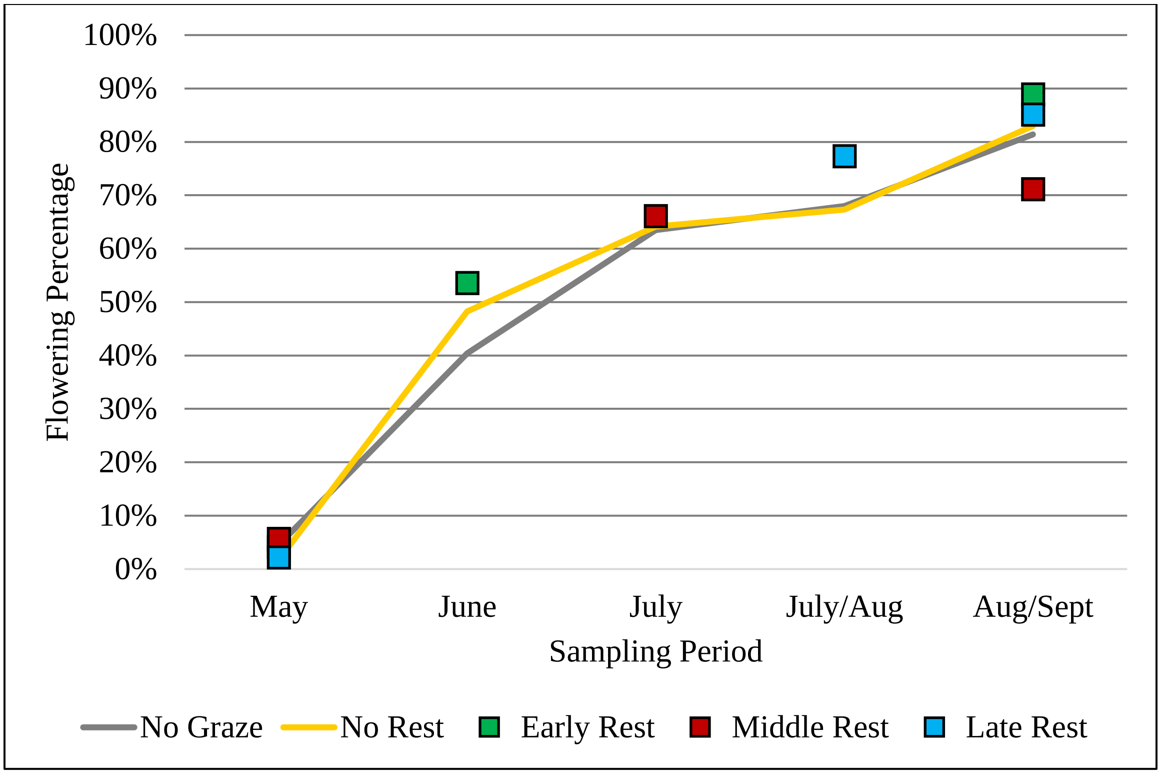

Figure 2.12. Flowering percentage of 10 native warm-season forb species pooled across years (2019-2020) at the same sampling periods in a big bluestem/indiangrass pasture for native grass pasture grazing experiment at the University of Tennessee – Northeast Tennessee AgResearch and Education Center, Greeneville, TN. †Flowering percentage compared across grazing treatments within sampling periods. All sampling periods compared no graze and no rest to the respective sampled grazing treatment. May = early rest, middle rest, and late rest; June = early rest; July = middle rest; July/Aug = late rest; Aug/Sept = early rest, middle rest, and late rest.

CONCLUSIONS

Switchgrass and BBIG plant persistence were not negatively impacted by interseeding CSA or warm-season forbs into SG and BBIG pastures. Each experiment indicated that plant density decline is more closely related to specific management practices and environmental conditions within a given year. For example, harvesting SG and BBIG hay multiple times within a season or overstocking pastures when grazing could reduce plant density. Incorporating CSA with NWSG is a viable option for producers to increase grazing days. Combining forbs and legumes with SG and BBIG has the ability to increase the overall forage quality and biodiversity of a pasture. When utilizing native legumes, DITI should be prioritized over ILBF and PPEA according to our results. Planting BESU in a mixture would allow for multiple blooms for at least the first two years while the perennial forbs establish. Based on plant abundance, persistence, observed flowering periods, and bloom abundance, planting BESU, DITI, LANC, MAXI, OXEY, and PCON in a mixture could allow for plant biodiversity while providing ample blooms during the NWSG grazing season. Since within-rest treatments did not affect forb plant density, rotational grazing does not seem to be a required grazing practice for stand persistence.

APPENDIX III

USING A BROWNTOP MILLET COMPANION CROP TO AID NATIVE GRASS ESTABLISHMENT

This appendix chapter is original work by Jonathan D. Richwine with contributions from co-authors Patrick D. Keyser, Dennis W. Hancock, and Amanda J. Ashworth. It has not yet been published but has been accepted for publication in "*Agronomy Journal*".

ABSTRACT

The lack of forage production during the seedling year is a barrier to wide-scale adoption of native warm-season grasses (NWSG). To address this, two NWSG establishment experiments were conducted in Knoxville, TN, 2016-2018, to determine the efficacy of big bluestem [BB; *Andropogon gerardii* Vitman] and switchgrass [SG; *Panicum virgatum* L.] establishment with browntop millet [BTM; *Urochloa ramosa* (L.) Nguyen] as a companion crop. Each experiment was a randomized complete block arranged as a 2x3 factorial. Two defoliation strategies [(1) harvests based on BTM maturity (boot to heading stage) for hay (HAY) or (2) clipping to control BTM competition by maintaining >50% sunlight reaching BB and SG seedlings (CLIP)] were coupled with three BTM seeding rates [0 (control), 11.2 (half-recommended rate), and 22.4 (full-recommended rate) kg pure live seed (PLS) ha^{-1}]. Only BTM seeding rate affected BB and SG plant density at dormancy. In all cases, the control had greater BB and SG plant density than the full-recommended rate, indicating that BTM impeded BB and SG establishment. All BTM seeding rates resulted in acceptable stands ($\geq$5.4 plants m^{-2}) of BB (both years) and SG (2017 only). Only the control allowed for acceptable stands of SG in 2016 (8.5 plants m^{-2}). Managing BTM for HAY produced a mean cumulative dry matter (DM) yield of 3.15 and 2.68 Mg ha^{-1} in 2016 and 2017, respectively. These findings show that BTM

can be a companion crop that helps offset production losses during BB and SG establishment.

INTRODUCTION

In recent years, considerable attention has been focused on NWSG such as BB and SG because of their potential contributions to forage for livestock (Tracy, Maughan, Post, & Faulkner, 2010; Burns & Fisher, 2013; Backus et al., 2017), biomass for bioenergy (McLaughlin & Kszos, 2005; Sanderson, Schmer, Owens, Keyser, & Elbersen, 2012), and integrated forage-biomass production systems (Guretzky, Biermacher, Cook, Kering, & Mosali, 2011; Mosali, Biermacher, Cook, & Blanton, Jr., 2013; Lowe et al., 2015; McIntosh et al., 2015). These grasses are desirable because of their drought tolerance (Sanderson & Reed, 2000; Buttrey et al., 2011), low input requirements (Vogel, Brejda, Walters, & Buxton, 2002; Boyer, Tyler, Roberts, English, & Larson, 2012; Kering, Butler, Biermacher, Mosali, & Guretzky, 2012), potential for achieving conservation goals (Gilley, Eghball, Kramer, & Moorman, 2000; Harper et al., 2015; West et al., 2016), and high resilience against climate variability (McLaughlin & Walsh, 1998; Owensby, Ham, Knapp, & Auen, 1999). Despite these many advantages, NWSG have not been widely re-adopted into production systems of the humid southeastern US.

One obstacle to integration of NWSG into forage and/or biomass production systems is stand establishment (Schmer et al., 2006; West & Kincer, 2011; Miesel, Renz, Doll, & Jackson, 2012), which likely is the greatest barrier for producer adoption of NWSG (Aiken & Springer, 1995; Parrish & Fike, 2005; Keyser, Schexnayder, Wilcox, Bates, & Boyer, 2021). Past researchers have identified competition control as a major contributor to failed establishment (McKenna, Wolf, & Lentner, 1991; Curran, Ryan, Myers, & Adler, 2011; Hedtcke, Sanford, Hadley, & Thelen, 2014). Past researchers have explored planting NWSG following a cool-season annual cereal cover crop to aid in NWSG establishment (Hedtcke et al., 2014; Keyser, Ashworth, Allen, and Bates, 2016a). In many situations, cover crops can double as companion crops and can offset establishment losses during the establishment of perennial plants and may reduce weed competition (Singh, Batish, & Kohli, 2003; Milchunas, Vandever, Ball, & Hyberg, 2011). At higher latitudes, cool-season annuals may serve as companion crops. For example, Jungers, Wyse, and Sheaffer (2015) seeded a NWSG polyculture with barley [*Hordeum vulgare* L.] and oat [*Avena sativa* L.] in Minnesota and found average plant density greater than 50 plants m^{-2} after harvesting the companion crop for forage in July or August. Similarly, Miesel et al. (2012), also working in the Upper Midwest of the U.S., evaluated an oat companion crop and reported that treatments using herbicides were more effective at reducing weed pressure and increasing yield of native grasses than treatments with the cool-season companion crop.

While prior experiments have focused on planting NWSG following or into cool-season annuals, research using warm-season companion crops has been limited to date. Warm-season annual plants can provide forage during the year of establishment. Hintz, Harmoney, Moore, George, and Brummer (1998), working in Iowa, successfully established both BB and SG with a corn [*Zea mays* L.] companion crop achieving stand densities typically in excess of 20 plants m^{-2}, well above thresholds required for production stands. Establishment in their study was successful irrespective of corn density or harvest date, but in all cases included atrazine [6-chloro-N-ethyI-*N'*-(lmethylethyl)-l,3,5-triazine-2,4-diamine], a product no longer labeled for native grass establishment. Similarly, Anderson et al. (2016), working in Illinois, reported SG stand densities that exceeded 20 plants m^{-2} when planted with a corn companion crop. Cossar and Baldwin (2002) found fall-recorded SG plant density to be inversely related to sorghum- [*Sorghum bicolor* (L.) Moench] sudangrass [*Sorghum bicolor* (L.) Moench ssp. *drummondii* (Nees ex Steud.) de Wet & Harlan] companion crop seeding rates in Mississippi. While they noted greater SG biomass yield when planted alone, Horton, Baldwin, and Cossar (2004) later reported no difference in SG biomass yield with respect to sorghum-sudangrass seeding rates when replicating the study at a different site.

Therefore, because of the paucity of published data, two NWSG experiments were implemented to investigate the potential of a warm-season annual companion crop, BTM, to aid in BB and SG establishment and provide harvestable forage in the establishment year. We hypothesized that by using BTM as the companion crop, its more diminutive stature relative to other commonly used summer annual forage crops and less robust regrowth following initial harvest would provide less competition to developing NWSG seedlings. Specifically, objectives were to evaluate BB (experiment 1) and SG (experiment 2) plant density and post-dormancy biomass yield (following the second year) based on (i) two BTM defoliation strategies and (ii) three BTM seeding rates.

MATERIALS AND METHODS

Site Description

Two NWSG (BB and SG) studies were conducted concurrently at the UTIA East Tennessee AgResearch and Education Center-Plant Science Unit (35°54'06.74"N, 83°57'27.11"W) in Knoxville, TN, from 2016-2017 (Site 1) and repeated at a second site on the same property during 2017-2018 (Site 2). The soil type for Site 1 was an Etowah silt loam (fine-loamy, siliceous, semiactive, thermic Typic Paleudults). This site previously grew turfgrasses, predominantly bermudagrass [*Cynodon dactylon* (L.) Pers.]. The soil type for Site 2 was dominated by Nonaburg channery silt loam (Clayey, mixed, active, thermic, shallow Inceptic Hapludalfs) with Heiskell silt loam (Fine-loamy, mixed, semiactive, thermic Aquic Hapludalfs) also being prevalent. This site had previously been planted in soybeans [*Glycine max* (L.) Merr.].

Experimental Design

Each experiment was a randomized complete block in a 2x3 factorial arrangement of treatments with four replicates. Treatment combinations of two defoliation strategies and three BTM seeding rates were assigned to 1.5 x 7.6 m plots. Defoliation strategies were (1) harvests based on BTM maturity (boot to heading stage) for hay (HAY) or (2) clipping to reduce BTM competition by maintaining >50% sunlight reaching BB and SG seedlings (CLIP). Both strategies were conducted when visual estimates met defoliation parameters. Browntop millet seeding rates were 0 (control), 11.2 (half-recommended rate), and 22.4 (full-recommended rate) kg PLS ha^{-1}. Browntop millet defoliations were conducted using a Carter forage harvester (Carter Manufacturing Company, Inc., Brookston, IN) at a 30.5-cm cutting height to reduce the probability of cutting developing BB or SG seedlings during the initial year of each experiment. Defoliation events are listed in Table 3A.1. For BB only, imazapic ((±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid) was also evaluated at a rate of 146 mL ha^{-1} a.i., a commonly used approach to competition control during establishment for this species. Imazapic was not included in any models or statistical analysis, but solely used for numerical comparison. Establishment locations differed (Site 1 vs. Site 2) to avoid confounding results caused by germination and emergence of dormant seed from the preceding year.

Both BB and SG were no-till drilled on 20 April 2016 and 11 May 2017 using an Almaco® (Nevada, IA) 8-row no-till plot drill following an application of glyphosate (N-[phosphonomethyl] glycine, isopropyl-amine salt) at a rate of 2.2 kg ha^{-1} a.i. Browntop millet was drilled perpendicular to BB and SG to minimize disturbance to BB or SG seed or emerging seedlings on 9 May 2016 and 1 June 2017. Due to an equipment malfunction in 2016, SG was replanted on 8 June 2016. The site was conventionally prepared and BTM reseeded on 21 June 2016. Big bluestem and SG (c.v. 'OZ 70' and 'Alamo', respectively; Bamert Seed Co., Muleshoe, TX) were drilled at 6.7 and 10.1 kg PLS ha^{-1}, respectively, at a 0.6-cm seeding depth. All plots in both experiments received 67.3 kg N ha^{-1} in the form of urea [$CO(NH_2)_2$] during the second growing-season only. Applying N fertilizer during the establishment year is not recommended for NWSG establishment to avoid accentuating weed competition (Keyser, Harper, Bates, Waller, & Holcomb, 2011). Nitrogen was the only macronutrient applied during all experiments.

Data Collection

Mean monthly air temperature and precipitation for each year were collected at a weather station located on ETREC (550-650 m from experiment locations) and compared to the 30-year means for that location (NOAA, 2020). Seedling counts for BB, SG, BTM, and weeds (both broadleaf and grass species) were conducted using a 0.45 x 0.45 m quadrat at five random areas per experimental unit. Then, plant density for each evaluated species was calculated. Plant density data were collected at 30 and 60 days after planting (DAP) BB or SG and only for BB and SG in mid-December of the first year – dormant period. Following the second growing season of each experiment, plots were harvested during dormancy (late November – early December) to obtain aboveground biomass yield (Mg ha^{-1}) using a Carter forage harvester with a 91.4-cm cutting width at a 20.3-cm cutting height (Ashworth et.al, 2015). In spring of the second year, we evaluated each experiment for the need for operational weed control. As stands had minimal competition at this point, no herbicides were deemed necessary, and none were applied. Thus, by the end of the second growing season, harvested biomass was clean and representative of normal production stands and no separations of crop and weed components were needed. Subsamples of BB and SG were collected from each plot at harvest, weighed, dried at 49°C in a forced-air oven (Wisconsin Oven Corporation, East Troy, WI) for at least 72 hours, and re-weighed to determine percent moisture (averaged 11% for BB and 21-25% for SG) for use in calculating dry matter (DM) yield (Ashworth et. al, 2015). Biomass yield is reported accordingly.

Statistical Analysis

Establishment-year plant density (BB, SG, weed, and BTM + weed m^{-2} for 30 and 60 DAP and BB and SG seedlings during dormancy) and second-year biomass DM yield data were analyzed under an ANOVA model in SAS v.9.4 (SAS Institute, 2013) using PROC MIXED to determine differences ($\alpha = 0.05$) among main effects and interactions.

Fixed effects were defoliation strategy and BTM seeding rate, and block was a random effect for each NWSG experiment. Defoliation strategy was not incorporated into 30 and 60 DAP establishment-year plant density statistical analysis since neither HAY nor CLIP had occurred prior to conducting these counts. Based on results from past studies (Keyser et al., 2016a; Keyser, Ashworth, Allen, & Bates, 2016b), and the potential influence from annual air temperature and timing differences, experimental years were analyzed separately for each study. All models were tested for normality of residuals using Shapiro-Wilk test ($W \geq 0.90$). Fisher's least significant difference was used for mean separations. Post-hoc regressions were conducted using PROC REG to determine the relationship between BTM + weed plant density and BB or SG plant density (at 30 and 60 DAP combined across sites), as well as for BB and SG plant density at dormancy and year two biomass DM yield for each site. These tests allowed us to explore potential relationships in competition that could affect establishment success, as well as minimum stand density thresholds for seedling-year stands.

RESULTS

Environmental Conditions

During the three years of the study, growing-season (April through September) mean monthly air temperatures remained near or above 30-year means (Figure 3A.1a). Monthly precipitation during May and June of all three years was similar to 30-year means (Figure 3A.1b). However, July through September were abnormally dry in 2016 while April and August in 2017 were atypically wet (75% and 74% greater than 30-year mean, respectively). August was then followed by a drier than normal September. In

2018, July through September received greater than or equal to 30-year mean amounts of rainfall (NOAA, 2020).

Big Bluestem

Establishment-year Plant Density

Plant density during dormancy of BB did not differ for either defoliation strategy at either site (Table 3A.2). However, BB establishment-year plant density differed by BTM seeding rate at 60 DAP and dormancy, but at Site 1 only (Table 3A.2), with control plots having the greatest BB plant density in both cases (Figure 3A.2 and 3A.3). At Site 1, establishment-year weed plant density did not differ at 30 DAP but was reduced where BTM was planted by 60 DAP (Table 3A.2). Browntop millet seeding rate affected BTM + weed establishment-year plant densities for both sites. At Site 1, the full-recommended rate had the greatest BTM + weed plant density at 30 DAP (342.5 seedlings m^{-2}) and 60 DAP (235.0 seedlings m^{-2}; Figure 3A.2). For Site 2, a compensatory effect on establishment-year weed plant density from BTM was not observed. Given that establishment-year weed plant density never differed at Site 2, it was apparent that BTM simply added to the overall level of competition without influencing BB plant density. Overall, there was a weak linear relationship between BB and BTM + weed establishment-year plant density at 30 (P = 0.013; r^2 = 0.13; m = -0.03 seedling seedling^{-1}) and 60 (P = 0.029; r^2 = 0.10; m = -0.02 seedling seedling^{-1}) DAP. When using imazapic, BB plant density at Site 1 and 2 (10.8 and 34.0 seedlings m^{-2}, respectively) was numerically greater than all BTM seeding rates (Figure 3A.3). Big bluestem establishment-year plant density across all BTM seeding rates was 7.8 and 17.1 seedlings m^{-2} for Site 1 and 2, respectively.

Biomass Dry Matter Yield

For second-year BB biomass DM yield, only BTM seeding rate at Site 1 was significant (Table 3A.3). Plots without BTM had the greatest yield (3.58 Mg ha^{-1}; Figure 3A.4a) while the half- and full-recommended BTM seeding rates had similar yields (2.35 and 1.96 Mg ha^{-1}, respectively). All BTM seeding rates produced similar BB yields at Site 2. Second-year biomass DM yield was positively related to BB establishment-year plant density at dormancy at Site 1 (P = 0.009; r^2 = 0.28; m = 0.104 Mg $seedling^{-1}$) and Site 2 (P = 0.017; r^2 = 0.23; m = 0.026 Mg $seedling^{-1}$).

Switchgrass

Establishment-year Plant Density

Browntop millet seeding rate influenced plant density of SG, weeds, and BTM + weeds at Site 1 and BTM + weeds at Site 2 (Table 3A.4). The 0 kg ha^{-1} BTM seeding rate had the greatest establishment-year plant density of BTM + weeds at 30 DAP (272 seedlings m^{-2}) at Site 1 and both 30 and 60 DAP (158 and 145 seedlings m^{-2}, respectively; Figure 3A.5) at Site 2. Browntop millet + weeds plant density for the half- and full-recommended BTM seeding rates did not differ at any of these times. Furthermore, SG plant density did not differ at 30 DAP at Site 1 or 30 and 60 DAP at Site 2. However, at 60 DAP for Site 1, BTM + weeds plant density as well as SG plant density were greater for the 0 kg ha^{-1} BTM seeding rate than either the half or full BTM seeding rates. Regression analysis for SG and BTM + weed establishment-year plant density at 30 DAP was not significant (P = 0.080) while there was a weak, positive relationship (P = 0.008; r^2 = 0.14; m = 0.15 seedling seedling^{-1}) at 60 DAP.

Biomass Dry Matter Yield

For second-year biomass DM yield of SG, defoliation strategy was only significant for Site 2 (Table 3A.3). Yield for HAY (2.69 Mg ha^{-1}) was slightly greater than CLIP (2.44 Mg ha^{-1}). However, both SG defoliation strategies only occurred once with HAY taking place ten days after CLIP (Table 3A.1). On the other hand, BTM seeding rate affected second-year biomass DM yield for both sites (Table 3A.3). For Site 1, plots without BTM had the greatest yield (5.10 Mg ha^{-1}; Figure 3A.4b). Where establishment-year plant densities were low at Site 1 in the presence of BTM, second-year biomass DM yields were reduced substantially relative to the control. At Site 2, only biomass DM yields for the control (2.67 Mg ha^{-1}) and full-recommended (2.40 Mg ha^{-1}) BTM seeding rate differed. There was a positive relationship between biomass DM yield and SG plant density during dormancy at Site 1 (P < 0.001; r^2 = 0.74; m = 0.306 Mg seedling^{-1}) but not Site 2 (P = 0.583).

Browntop Millet

Across both experiments (BB and SG), HAY produced a BTM mean cumulative DM yield of 2.92 ± 0.27 Mg ha^{-1} (half-recommended seeding rate) and 3.37 ± 0.29 Mg ha^{-1} (full-recommended seeding rate) at Site 1. Yields at Site 2 were comparable and resulted in 2.72 ± 0.48 Mg ha^{-1} (half-recommended seeding rate) and 2.64 ± 0.43 Mg ha^{-1} (full-recommended seeding rate).

DISCUSSION

To date, only four published studies (Hintz et al., 1998; Cossar & Baldwin, 2002; Horton et al., 2004; Anderson et al., 2016) addressed the use of a warm-season annual companion crop, with two having used corn and two sorghum-sudangrass. Our studies showed contrasting results when evaluating BTM as a companion crop for BB and SG. Yields of BTM were less than those (4.3-10.4 Mg ha^{-1}) presented by McLaughlin, Fairbrother, and Rowe (2004) when seeding a BTM monoculture using the full-recommended rate. However, producers could benefit from forage production during BB and SG establishment by using the half-recommended BTM seeding rate since BTM yield loss was negligible between the full- and half-recommended rates. If producers do not need to compensate for lost forage production during the BB or SG establishment year, then not planting BTM will likely result in denser stands of BB or SG.

Establishment-year Plant Density

Big bluestem establishment-year plant density at dormancy was not influenced by defoliation strategy. At Site 1, BB was harvested twice for both defoliation strategies with only six days separating the first harvest for each. This narrow window, which was a result of the rapid development of the BTM at this time of year and an inability to implement CLIP due to rainfall and field conditions, precluded any meaningful advantage from CLIP. At Site 2, only one defoliation occurred on all BB and SG plots with all BB plots harvested on the same day. Persistent rains delayed planting BTM at Site 2 in spring 2017 while allowing an abundant weed population to develop. As a result, BTM was slow to develop because of the heavy weed pressure already in place. Because of the lack of regrowth of the BTM following the initial defoliation for both defoliation strategy treatments, no additional harvests were implemented at Site 2. Thus, there would not have been an expectation that HAY or CLIP would influence plant density, especially at Site 2. Clearly, timing of planting BTM relative to BB planting and timing of HAY and CLIP were sensitive and critical factors with this system.

In the case of SG at Site 1, the earlier harvest date for the initial CLIP defoliation preceded the first HAY harvest by 21 days. Nevertheless, SG establishment-year plant density was not improved by this harvest interval. Given the later SG planting date at Site 1, the timing of the initial CLIP defoliation may have been too soon after BTM planting to be beneficial. Furthermore, the rapid development of BTM between the first and second CLIP could have been substantial enough that the 12 Aug 2016 harvests occurred after the BTM had already suppressed the SG seedlings. This underscores the importance of timing in such CLIP defoliations. It also may suggest that there is a critical point in seedling development that occurs between 30 and 60 DAP as was apparent for BB in the context of BTM seeding rate.

When BTM seeding rate affected BB and SG density, unplanted controls had greater BB and SG density than the BTM companion crop. Likewise, Cossar and Baldwin (2002) reported greater end-of-season SG plant density when planted alone than with a sorghum-sudangrass companion crop. Anderson et al. (2016) also found that SG plant density was greater when SG was established alone as compared with a corn companion crop in Illinois. In contrast, Hintz et al. (1998) found reduced post-dormancy plant density in the first year of their study when BB was planted alone versus with a corn + atrazine companion crop. However, in the second year of their study, there was no difference in plant density based on these treatments.

In the current study, there was also a lack of consistency between sites with respect to SG plant density. At Site 1, the use of BTM appeared to increase competition at 30 DAP (Figure 3A.5). Regardless, this competition did not influence SG plant density at this stage of stand development. Yet by 60 DAP, SG density for both half- and full-recommended BTM seeding rates was reduced to 50% or less of that of the control. The greater SG density in the control (30 seedlings m^{-2}) at 60 DAP suggests that BTM presented more effective competition than weeds to SG seedlings. The more effective competition of the BTM at Site 1 compared to Site 2 was likely the result of the later planting date of SG at Site 1 due to the initial stand failure. In any case, SG plant densities for the half- and full-recommended (1.9 seedlings m^{-2} for both) BTM seeding rates at Site 1 during dormancy were well below desirable targets for production while those at Site 2 were more than adequate.

At Site 1, BTM had the desired effect of suppressing weed populations for BB at 60 DAP. In this case though, BB seedlings, which had not been suppressed at 30 DAP became so by 60 DAP. This suggests that the negative impact of the additional competition (i.e., light and space) from the BTM did not become a factor until the BTM canopy had become more developed at 60 DAP. Also, the lack of any difference in BB plant density between the full- and half-recommended BTM seeding rates at 60 DAP at Site 1 may have been because a threshold was possibly reached using half-recommended rate. Therefore, any additional competition from the full BTM seeding rate had no additional impact.

At Site 2, the competition from BTM on BB seedlings was negligible likely because of the late start for BTM and the already heavy weed pressure. That the patterns observed for BB density at 60 DAP carried through to dormancy (Figure 3A.3) at both sites suggests stand development may be largely determined by 60 DAP. The lack of a stronger linear relationship between BB and BTM + weed plant densities at 30 and 60 DAP may have been due to the variability in BTM stand development and, in turn, its influence on BB seedling recruitment. The half- and full-recommended BTM seeding rates at Site 1 produced BB plant densities at dormancy below the target threshold of 10 plants m^{-2}. Using imazapic allowed for greater BB plant density than all BTM seeding rates at both sites. This finding further reinforces the impact of competition on seedling recruitment.

For both species examined, negative effects of competition were more apparent at 60 DAP than at 30 DAP suggesting an important stage in stand development. Indeed, patterns apparent at 60 DAP carried through to fall dormancy for both sites. Browntop millet appeared to have been more problematic for competition than the weeds. This was borne out by the fact that when BTM development was limited at Site 2, SG densities were well above target plant densities, regardless of BTM seeding rate. Moreover, BTM at Site 2 appeared only to provide additive competition for BB, having no effect on weed plant density during the establishment year.

Biomass Dry Matter Yield

Big bluestem biomass DM yields were comparable (3.83 Mg ha^{-1}) to those reported by Rushing, Lemus, White, Lyles, and Thornton (2019) when harvesting second-year BB stands in Mississippi. At both sites, BB yield exhibited the same pattern as plant density at dormancy following the establishment year. The regression relationship only explained a modest amount of the variability in yield at either site. Previously, Keyser et al. (2016a) observed a relationship between establishment-year plant density and second-year yield in SG, but also found that there was great variability. They attributed this to density-dependent responses of individual plants and their ability to produce larger and more tillers. This same plant density-dependent process may be important for BB as well. The variability in the plant density-yield relationship may also be a function of the level of competition within a given plot based on weed size and/or density. These differences did not appear to be particularly influenced by variability in air temperature or rainfall between years, at least for treatments that included BTM, because yields for both the half- and full-recommended BTM seeding rates were similar at both sites.

Switchgrass biomass DM yields were also similar to those reported in previous studies, ranging from 4.0-8.0 Mg ha^{-1} (Hedtcke et al., 2014; Keyser et al., 2016a, 2016b). Yields from the SG experiment were consistent with those reported by Cossar and Baldwin (2002). However, yields were contrary to those later reported by Horton et al. (2004) which found no difference in SG biomass yield when replicating the Cossar and Baldwin (2002) study. Keyser et al. (2016b) noted SG biomass yield increased until a threshold of 8 plants m^{-2} and plateaued at densities beyond 10 plants m^{-2}. Similarly, in the current study, SG biomass DM yield increased until reaching 8 plants m^{-2} at Site 1 and plateaued beyond 10 plants m^{-2} at Site 2. The concept of stocking threshold for yield was further reinforced by the sizeable difference in biomass DM yield for the unplanted controls between Site 1 (5.10 Mg ha^{-1}) and Site 2 (2.67 Mg ha^{-1}). Despite the five-fold greater number of seedlings at Site 1 (42.9 seedlings m^{-2}) than at Site 2 (8.5 seedlings m^{-2}), yields were only 1.9 times greater. Although this may have been the result of factors other than SG plant density, it may also suggest a plant density-dependent threshold for SG plant population. Another reasonable explanation is that the plants in the Site 2 control were not as individually vigorous or well developed as those from Site 1. Mean monthly air temperature and total monthly precipitation, which were greater than the 30-year mean for April and May for Site 2, may have moderated the difference in yield between the two sites. Lee and Boe (2005) found a strong linear relationship between maximum SG biomass production and April through May precipitation in South Dakota.

CONCLUSIONS

Defoliation strategy did not affect BB or SG seedling establishment. Timelier implementation of canopy treatments may have had a greater impact on the results. Furthermore, the rapid growth rate of the well-established BTM at Site 1 made more precise timing of treatments difficult. Conversely, the lack of a consistent effect from defoliation strategies may suggest producers could have some flexibility in implementing these treatments. All BTM seeding rates resulted in acceptable stands ($\geq$ 5.4 plants m^{-2}; Keyser et al., 2011) of BB at Site 1 and both BB and SG at Site 2, whereas only the control allowed for acceptable stands of SG (8.5 $\pm$ 2.1 plants m^{-2}) at Site 1. Timing of BTM plantings and precipitation patterns appear to be an important consideration for using this species as a companion crop for improving BB or SG establishment. Precipitation between seeding the NWSG and BTM at Site 2 was greater than that at Site

1 leading to substantially greater weed germination prior to emergence of BTM at Site 2. Thus, Site 1 BTM stands were more developed and appeared to be more competitive with weed seedlings. At Site 2, on the other hand, weeds were well developed by the time BTM seedlings emerged in large numbers, thus reducing BTM vigor. Regardless, lower BTM seeding rates produced greater BB and SG dormancy plant density and greater second-year biomass DM yields at Site 1, but not at Site 2.

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APPENDIX IIIA

Table 3A.1. Harvest dates for browntop millet defoliation strategies for big bluestem and switchgrass at each site at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN, during establishment experiments in 2016 and 2017.

	Site 1		Site 2	
BTM Defoliation Strategy†	Big bluestem	Switchgrass	Big bluestem	Switchgrass
HAY	27-Jun 22-Jul	12-Aug	10-Jul	20-Jul
CLIP	21-Jun 22-Jul	22-Jul 12-Aug	10-Jul	10-Jul

†Browntop millet (BTM) defoliation strategy [HAY = harvests based on BTM maturity (boot to heading stage) for hay or CLIP = clipping to reduce BTM competition by maintaining >50% sunlight reaching big bluestem and switchgrass seedlings]

Table 3A.2. Mixed-effects ANOVA model results for establishment-year plant density of big bluestem seedlings, weeds, and browntop millet (BTM) + weeds at each site at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN, 2016-2017, during a big bluestem establishment experiment.

	Site 1						Site 2					
	30 DAP†		60 DAP		Dormancy		30 DAP		60 DAP		Dormancy	
Effect	*F* value§	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
Big bluestem												
HARV‡	-	-	-	-	1.82	0.197	-	-	-	-	2.00	0.178
RATE	0.07	0.937	7.68	**0.022**	4.36	**0.032**	0.72	0.523	0.79	0.495	1.80	0.200
HARV x RATE	-	-	-	-	3.06	0.077	-	-	-	-	0.74	0.495
Weeds												
RATE	2.45	0.167	9.93	**0.013**	-	-	0.55	0.603	0.16	0.859	-	-
BTM + Weeds												
RATE	74.48	**<0.001**	12.99	**0.007**	-	-	16.41	**0.004**	6.84	**0.028**	-	-

† Establishment-year plant density at 30 and 60 days after planting (DAP) of big bluestem and big bluestem plant density during dormancy. Since both BTM defoliation strategy treatments had not been conducted prior to 30 and 60 DAP seedling counts, HARV was not incorporated into the model as a dependent variable.
‡ HARV = BTM defoliation strategy (harvested for hay, harvested for competition control); RATE = BTM seeding rate (0, 11.2, and 22.4 kg PLS ha^{-1})
§ df num/den = Big bluestem – HARV 1/15, RATE 2/15, HARV x RATE 2/15; Weeds – RATE 2/6; BTM + Weeds – RATE 2/6

Table 3A.3. Mixed-effects ANOVA model results for big bluestem and switchgrass second-year biomass dry matter yield for each site at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN, during establishment experiments. Harvests were conducted in 2017 and 2018 for Site 1 and 2, respectively.

	Big bluestem				Switchgrass			
	Site 1		Site 2		Site 1		Site 2	
Effect	*F* value‡	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
HARV†	0.29	0.596	0.88	0.362	0.75	0.400	8.22	**0.012**
RATE	12.27	**<0.001**	1.77	0.205	29.99	**<0.001**	3.70	**0.049**
HARV x RATE	0.05	0.953	0.29	0.751	0.07	0.931	3.07	0.076

† HARV = browntop millet (BTM) defoliation strategies (harvest for hay, harvest for competition control); RATE = BTM seeding rate (0, 11.2, and 22.4 kg PLS ha^{-1})

‡ df num/den = HARV, 1/15; RATE, 2/15; HARV x RATE, 2/15

Table 3A.4. Mixed-effects ANOVA model results for establishment-year plant density of switchgrass seedlings, weeds, and browntop millet (BTM) + weeds at each site at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN, 2016-2017 during a switchgrass establishment experiment.

	Site 1						Site 2					
	30 DAP†		60 DAP		Dormancy		30 DAP		60 DAP		Dormancy	
Effect	*F* value§	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*	*F* value	*P* > *F*
Switchgrass												
HARV‡	-	-	-	-	0.05	0.832	-	-	-	-	1.70	0.212
RATE	0.46	0.653	6.88	**0.028**	8.22	**0.004**	0.33	0.729	3.13	0.117	0.88	0.435
HARV x RATE	-	-	-	-	0.53	0.599	-	-	-	-	0.18	0.835
Weeds												
RATE	8.48	**0.018**	43.46	<**0.001**	-	-	1.08	0.396	1.19	0.368	-	-
BTM + Weeds												
RATE	13.01	**0.007**	6.25	**0.034**	-	-	12.77	**0.007**	13.95	**0.006**	-	-

† Establishment-year plant density at 30 and 60 days after planting (DAP) of switchgrass and switchgrass plant density during dormancy. Since both BTM defoliation strategy treatments had not been conducted prior to 30 and 60 DAP seedling counts, HARV was not incorporated into the model as a dependent variable.

‡ HARV = BTM defoliation strategy (harvested for hay, harvested for competition control); RATE = BTM seeding rate (0, 11.2, and 22.4 kg PLS ha^{-1})

§ df num/den = Switchgrass – HARV 1/15, RATE 2/15, HARV x RATE 2/15; Weeds – RATE 2/6; BTM + Weeds – RATE 2/6

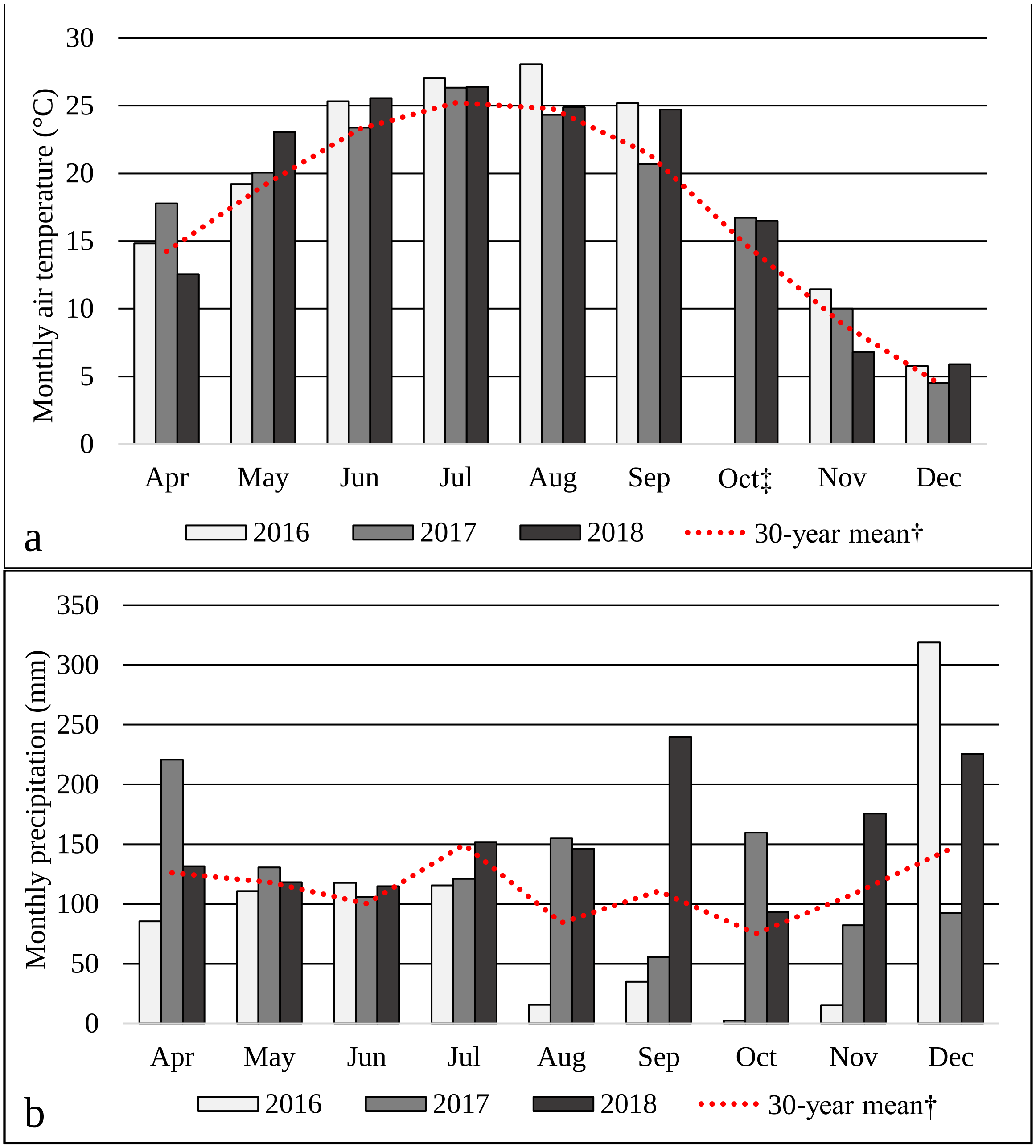


Figure 3A.1. (a) Mean monthly air temperature (°C) and 30-year mean and (b) total monthly precipitation (mm) and 30-year mean for East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN, 2016-2018. †Some months' data are missing in overall 30-year mean from 1988-2018. ‡No data were reported in 2016.

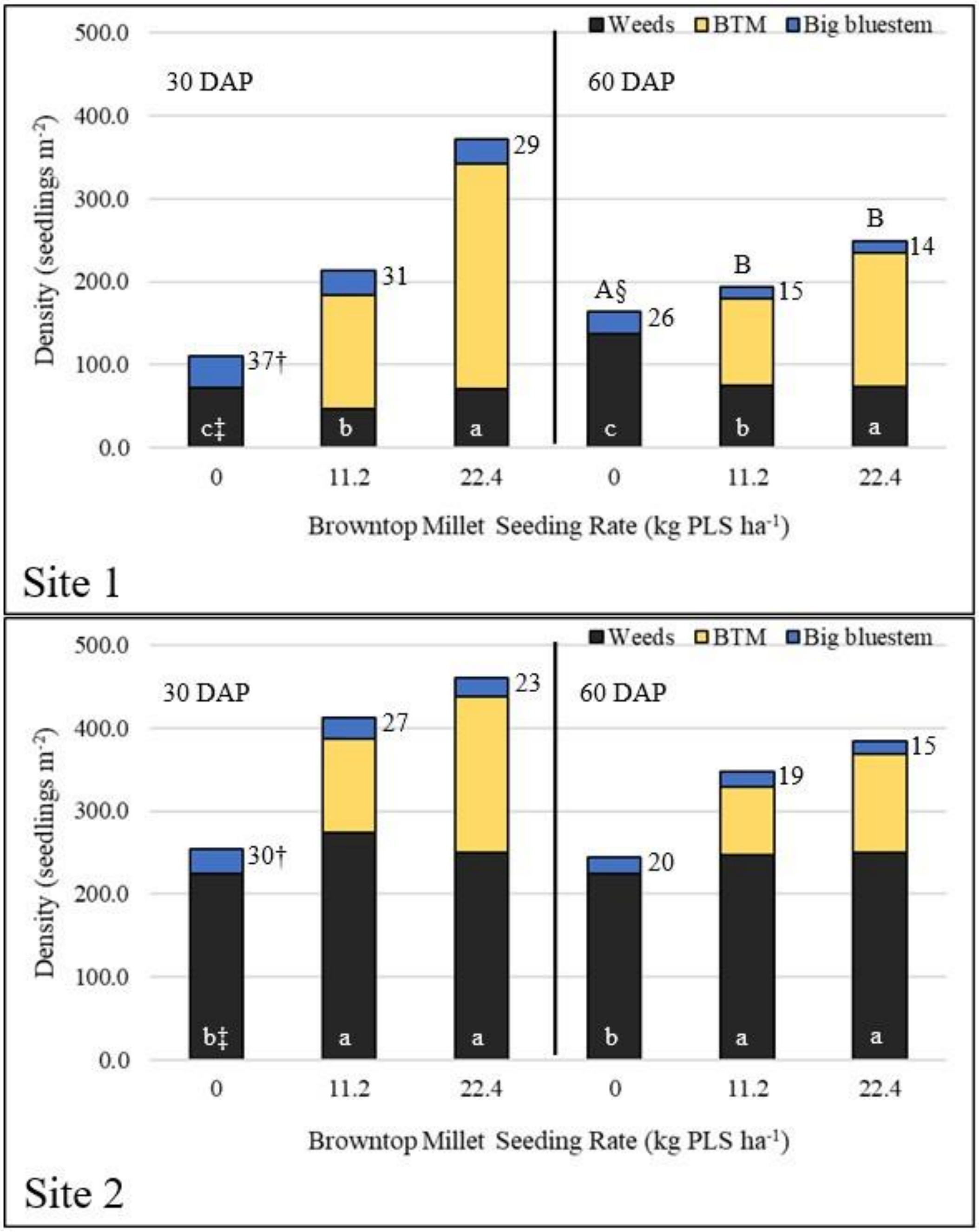


Figure 3A.2. Establishment-year plant density (seedlings m^{-2}) for big bluestem (BB), browntop millet (BTM), and weeds by BTM seeding rate (kg PLS ha^{-1}) at 30 and 60 days after planting (DAP) BB for Site 1 (top) and Site 2 (bottom) at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN. †Number of BB seedlings per BTM seeding rate at 30 and 60 DAP. ‡Different lowercase letters indicate significant differences among weed + BTM seedling totals by BTM seeding rates at 30 and 60 DAP within site. §Different UPPERCASE letters indicate significant differences for BB seedlings by BTM seeding rate at 30 and 60 DAP within site.

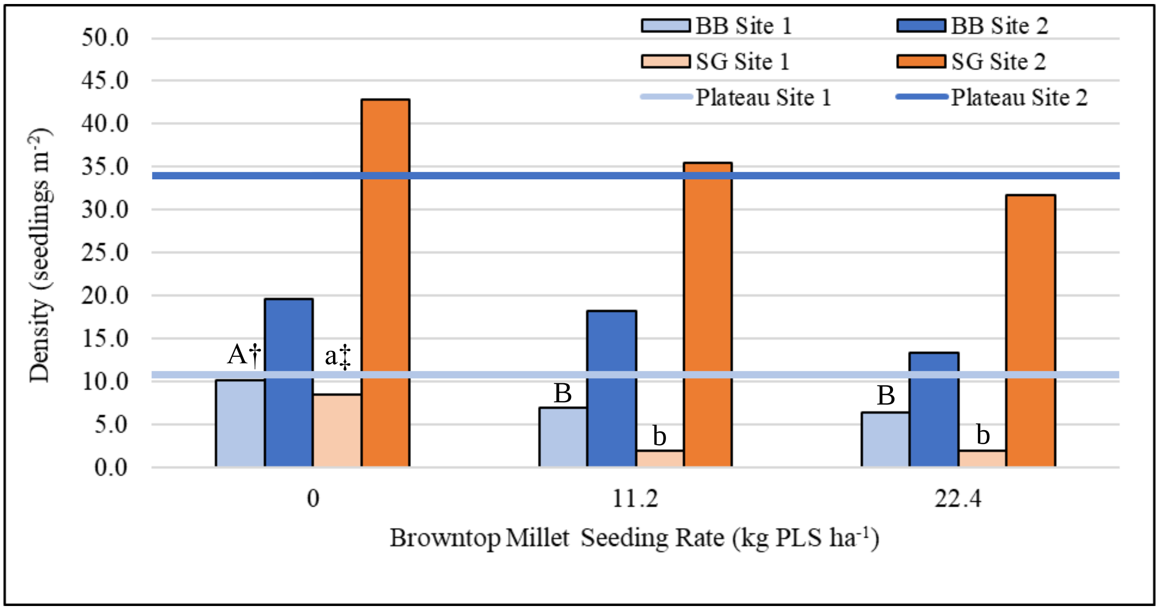


Figure 3A.3. Establishment-year plant density (seedlings m^{-2}) at dormancy for big bluestem (BB) and switchgrass (SG) by browntop millet (BTM) seeding rate (kg PLS ha^{-1}) compared to using imazapic (Plateau) for Site 1 (2016) and Site 2 (2017) at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN. Plant density for imazapic treatment (BB only) are horizontal lines; not compared statistically to other BB treatments. †Different UPPERCASE letters indicate significant differences for BB plant density by BTM seeding rate within site. ‡Different lowercase letters indicate significant differences for SG plant density by BTM seeding rate within site.

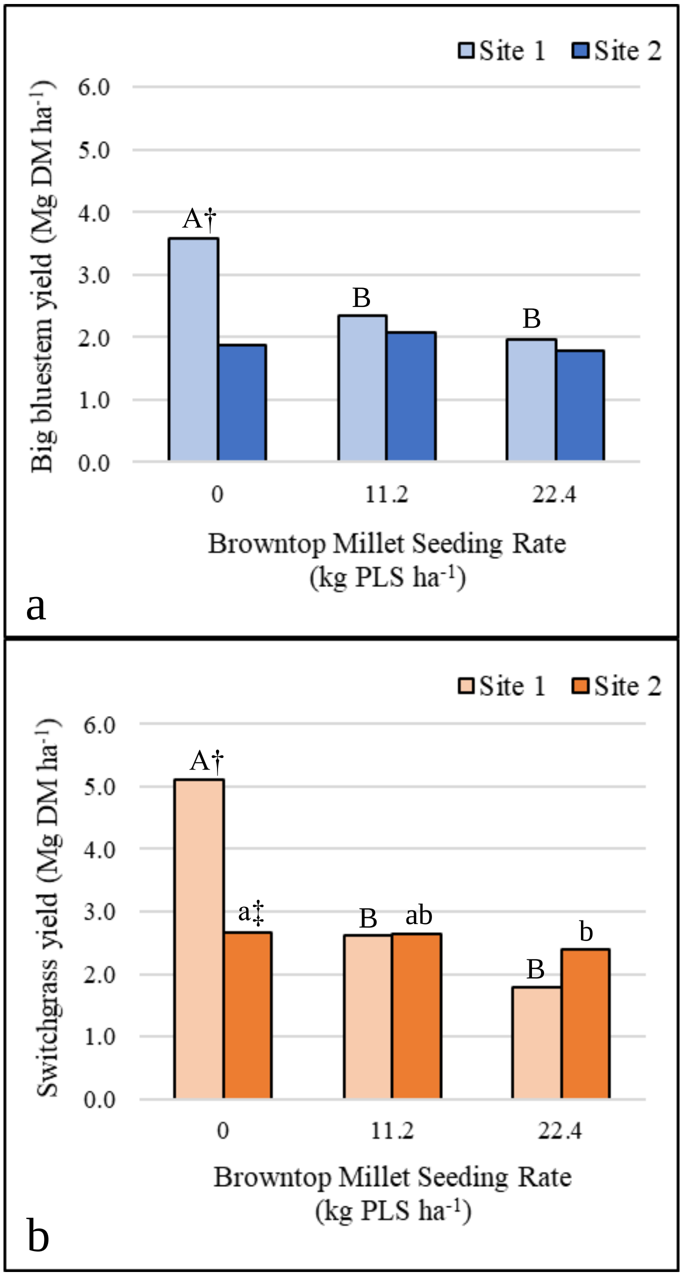
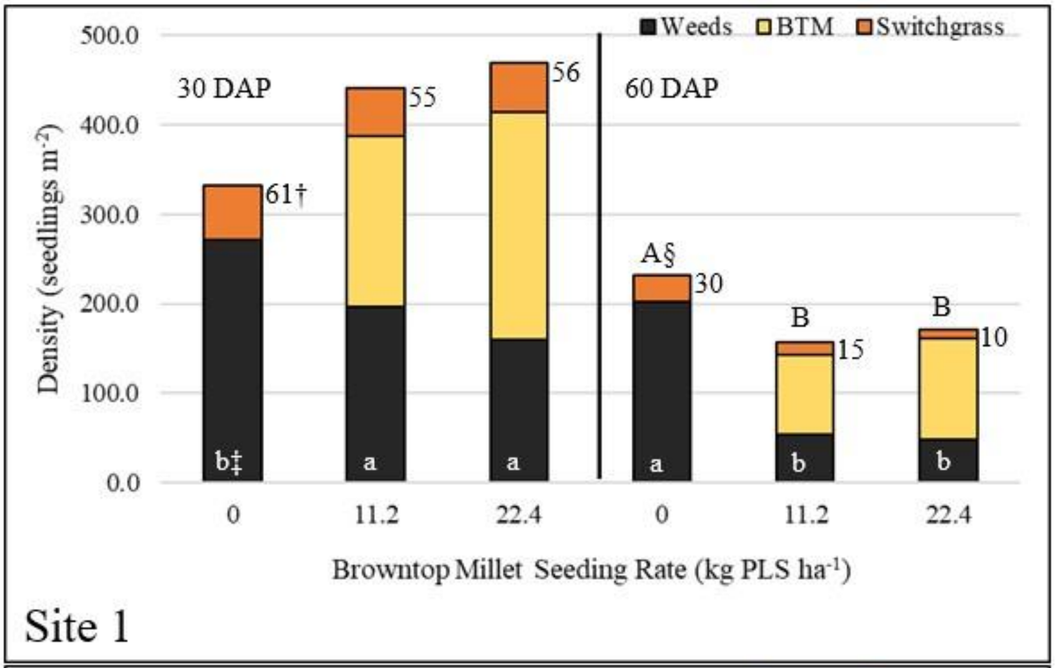


Figure 3A.4. Biomass dry matter (DM) yield (Mg DM ha^{-1}) for (a) big bluestem and (b) switchgrass by browntop millet (BTM) seeding rate (kg PLS ha^{-1}) following the second year of each study at Site 1 (2017) and Site 2 (2018) at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN. †Different UPPERCASE letters indicate significant differences among Site 1 (2017) DM biomass yield among BTM seeding rate per species. ‡Different lowercase letters indicate significant differences among Site 2 (2018) DM biomass yield among BTM seeding rate per species.



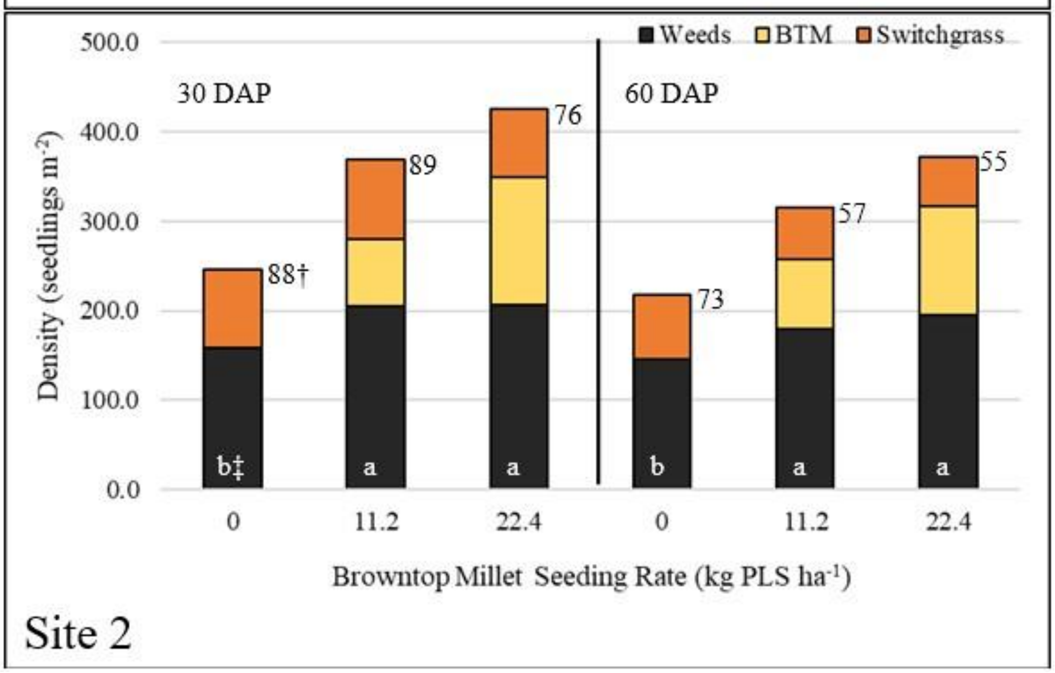


Figure 3A.5. Establishment-year plant density (seedlings m^{-2}) for switchgrass (SG), browntop millet (BTM), and weeds by BTM seeding rate (kg PLS ha^{-1}) at 30 and 60 days after planting (DAP) SG for Site 1 (top) and Site 2 (bottom) at East Tennessee AgResearch and Education Center-Plant Science Unit, Knoxville, TN. †Number of SG seedlings per BTM seeding rate at 30 and 60 DAP. ‡Different lowercase letters indicate significant differences among weed + BTM seedling totals by BTM seeding rate at 30 and 60 DAP within site. §Different UPPERCASE letters indicate significant differences for SG seedlings by BTM seeding rate at 30 and 60 DAP within site.

VITA

Jonathan D. Richwine was born and raised in Martin, TN. He graduated from Westview High School in Martin in 2004. He then attended the University of Tennessee – Martin, where he received a Bachelor of Science in Agriculture in 2009. He then received his Master of Science in Plant and Soil Science at Mississippi State University in 2016. During and between both degrees he worked with production agricultural. He aided farmers in baling and loading straw and worked part-time/seasonal positions at Helena Chemical Co. and Tennessee Crop Improvement Association, where he was introduced to chemicals, fertilizers, and crop traits commonly used in agriculture. His studies and research at Mississippi State focused on native grass breeding, biomass crop production, oil seed production, and forage production for grazing animals and hay; all of which intrigued me. Working with forages allowed him to be exposed to a different side of agriculture that he was not accustomed. He began his Doctor of Philosophy in Natural Resources at University of Tennessee – Knoxville in 2016. His research concentrated on land management from a forage producer standpoint but also from a wildlife conservationist point of view. Incorporating multiple flora species within a pasture system can supply various organisms with ample sustenance, from the bacteria and fungi in the soil profile to pollinators, rabbits, quail, and turkeys feeding and nesting in the same area.